

CELLULAR

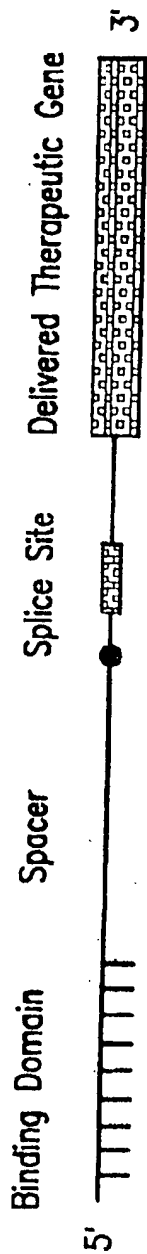
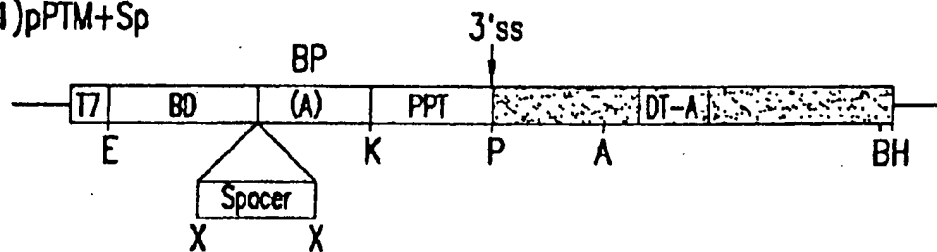


FIG. 1A

(1)pPTM+Sp



(2)pPTM+Sp

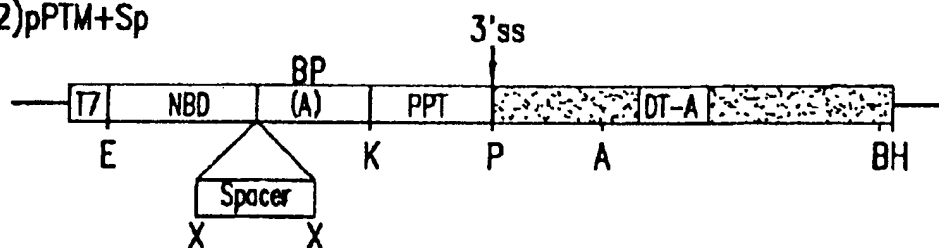


FIG.1B

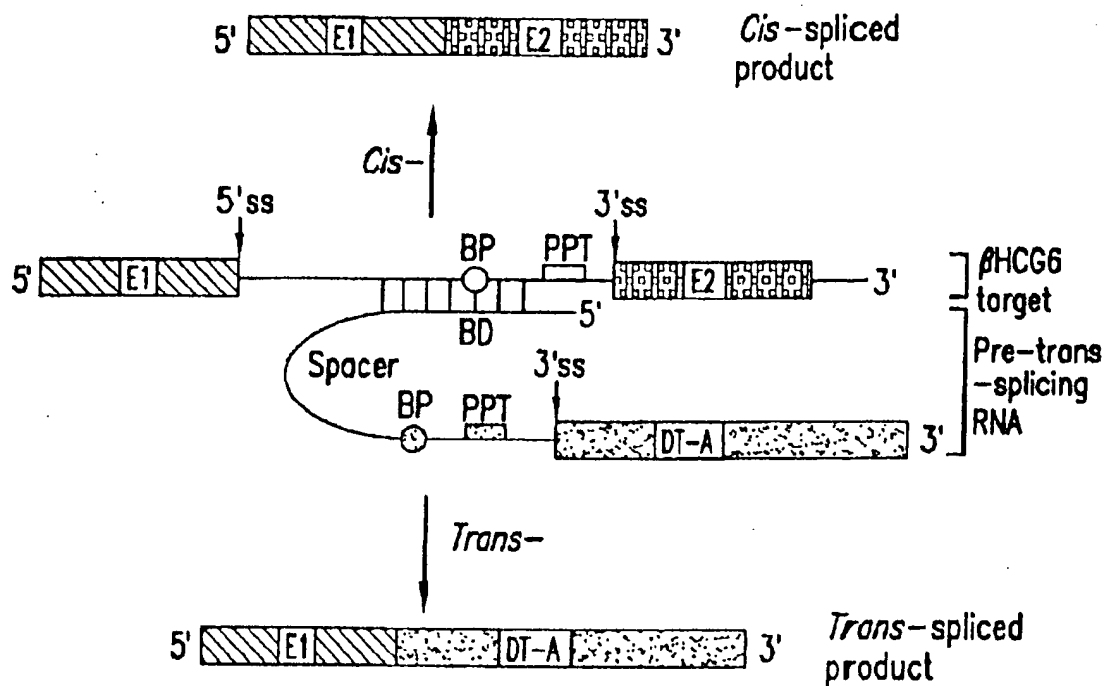


FIG.1C

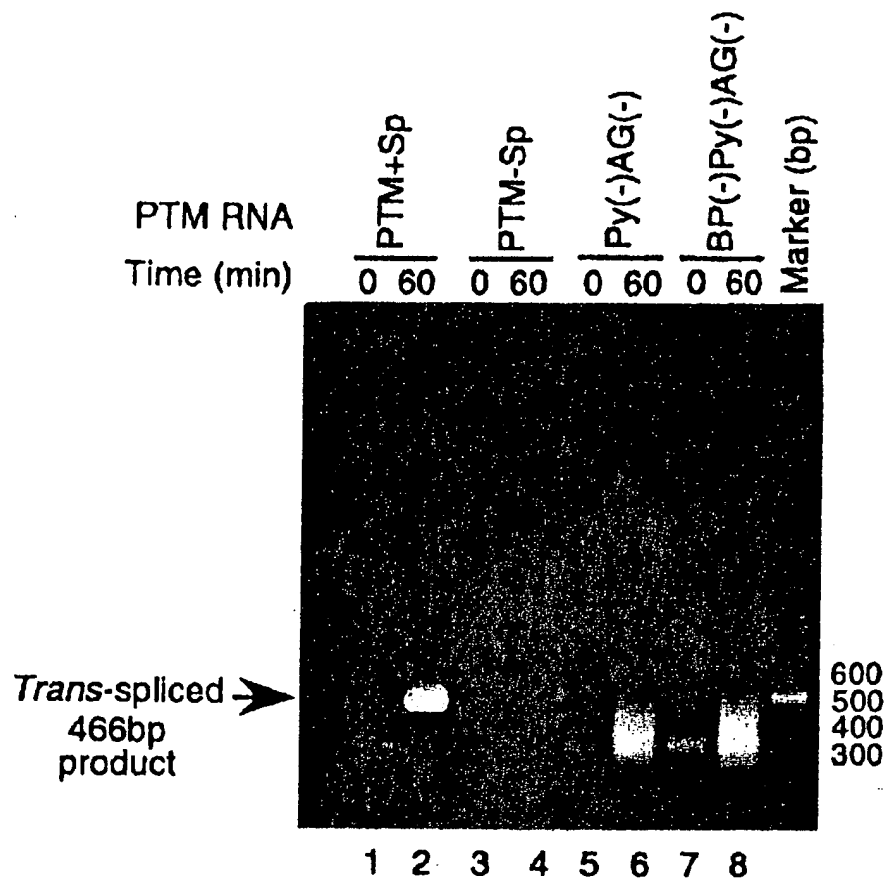


FIG.2A

**FIG. 2B**

00000000-010000

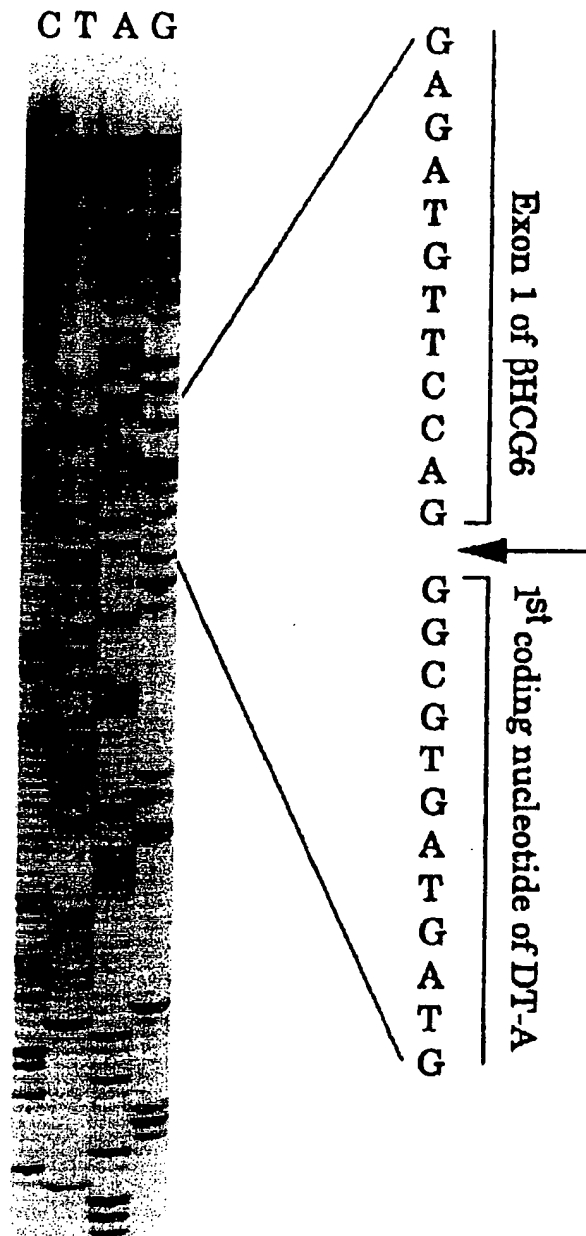
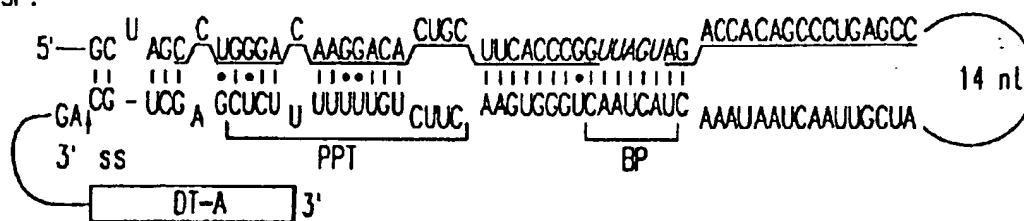
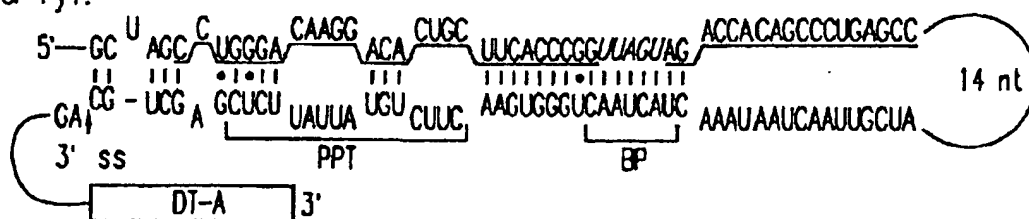


FIG.3

1. PTM+SF:



2. PTM+SF-Py1:



3. PTM+SF-Py2:

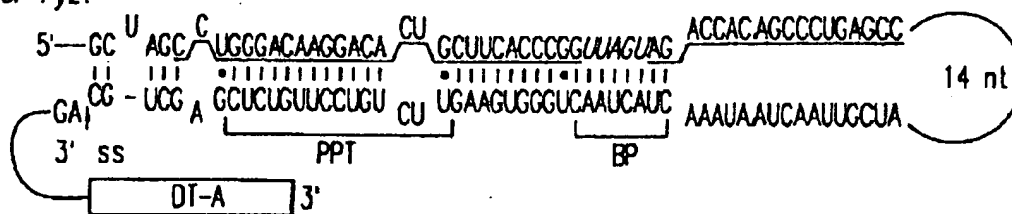


FIG.4A

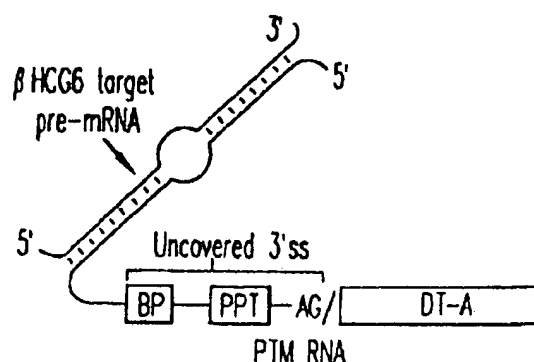


FIG.4B

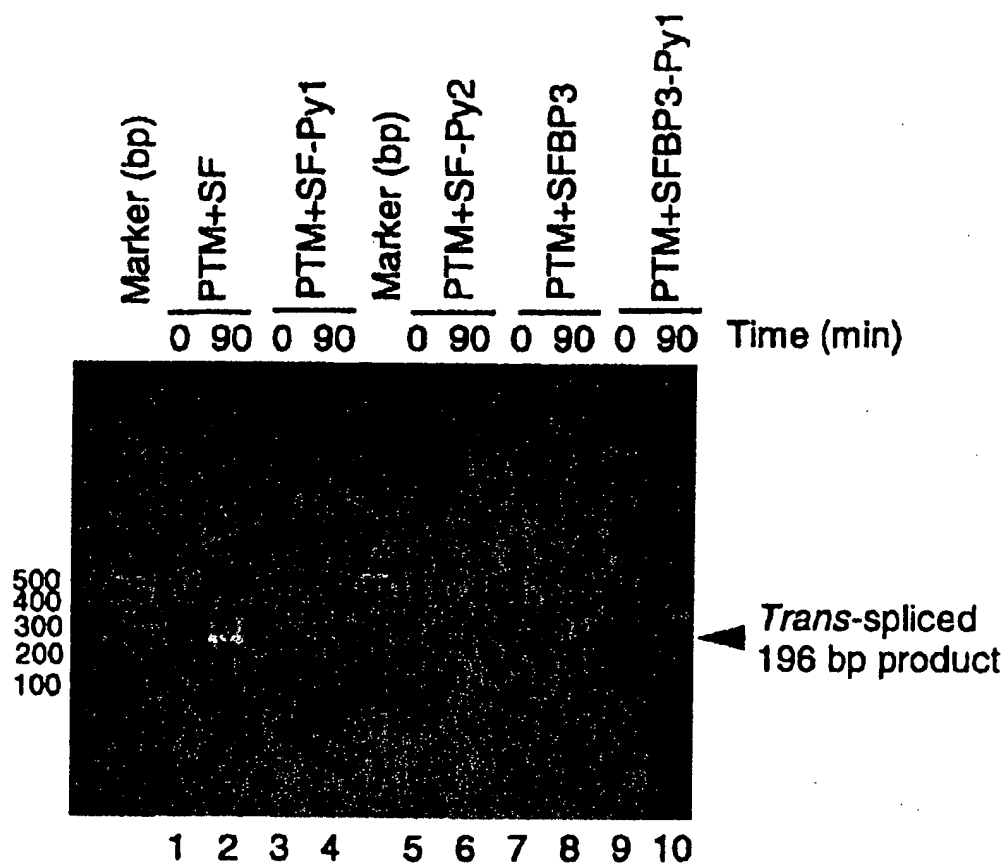


FIG.4C





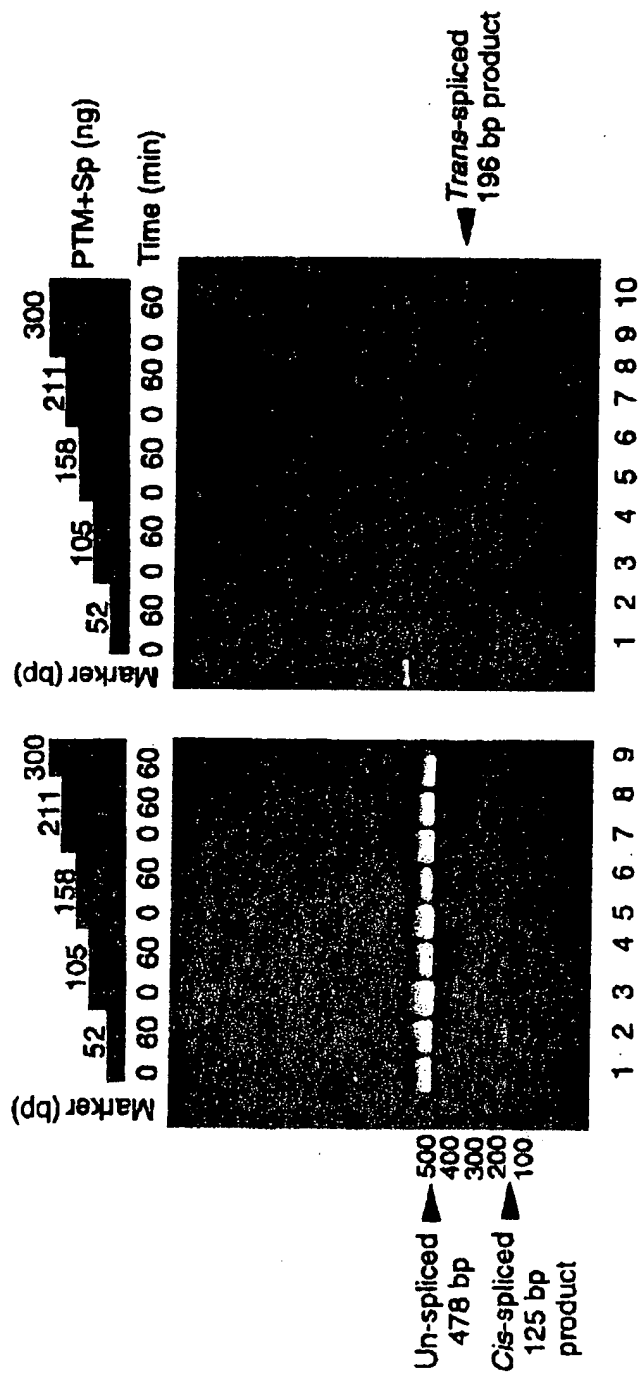


FIG. 6A

FIG. 6B

20070707 03002000

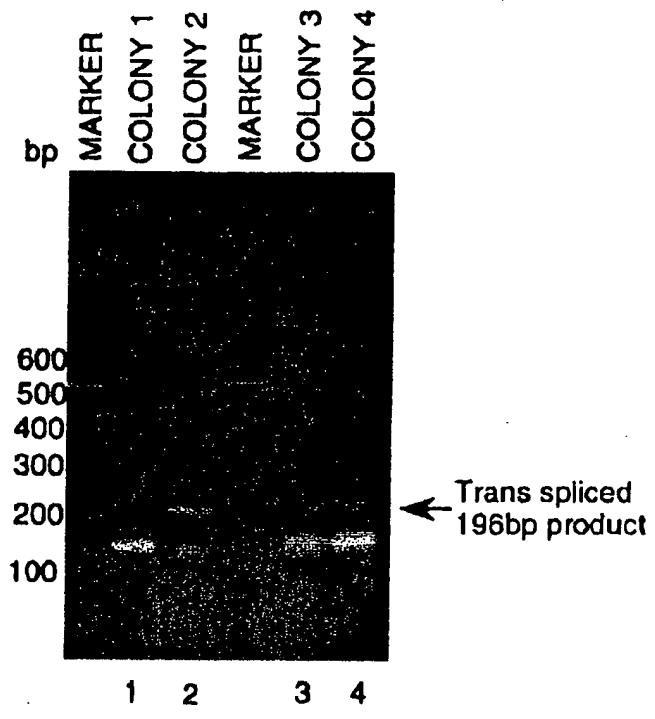


FIG.7A

P. 15

EXON 1 OF  $\beta$ HCG6 ↑  
 5'-CAGGGACGCACCAAGGATGGAGATGTTCCAG-GGGCTGATGATGTTGTT  
 ↑ 1ST CODING NUCLEOTIDE OF DT-A  
 GATTCTTCTTAATCTTTTGATGGAAACTTTTCTTCGTACCAACGGGACTA  
 AACCTGGTTATGTAGATTCCATTCAAAA-3'

**FIG. 7B**

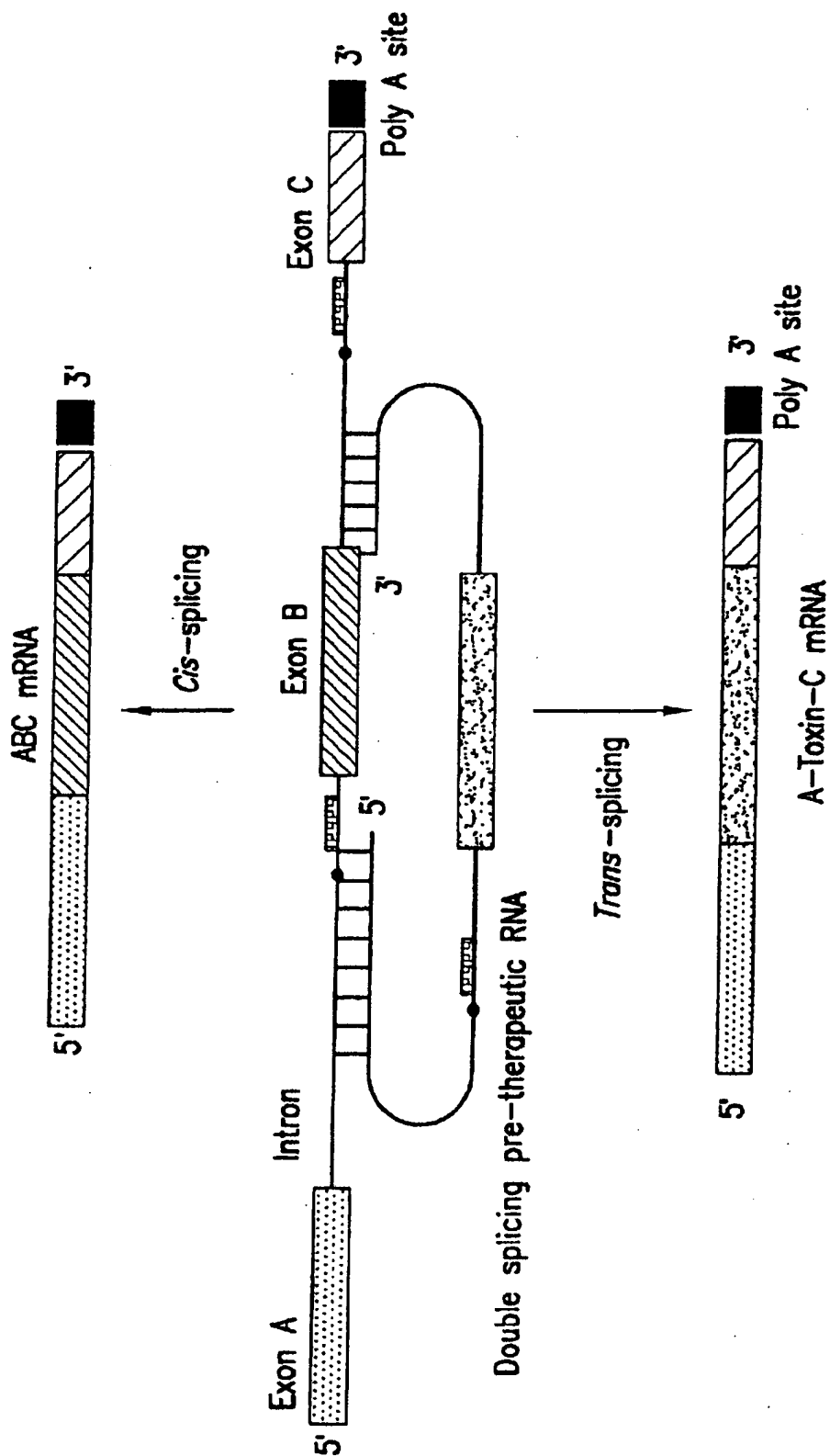
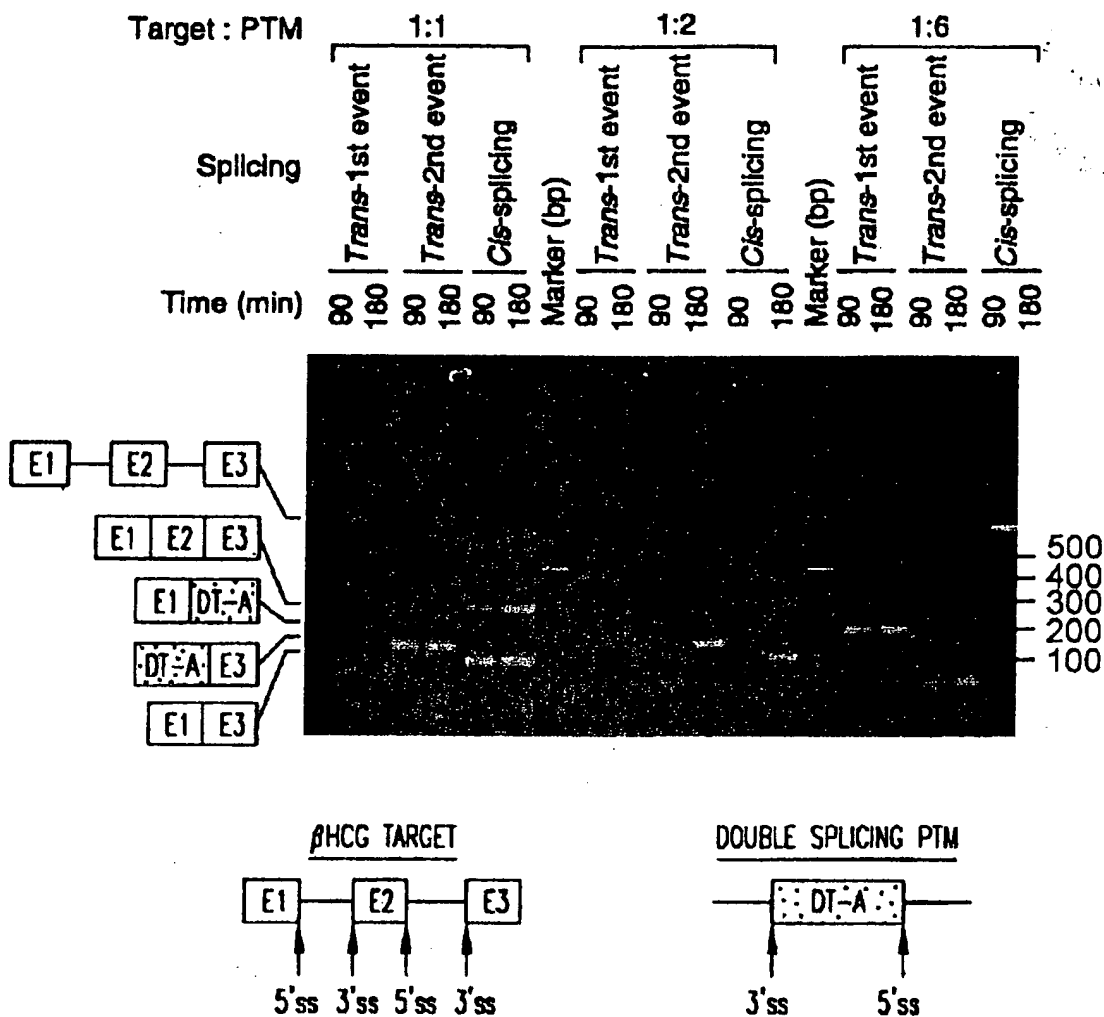


FIG.8A



Cis-spliced products

E1 E2 E3 = NORMAL *cis*-SPLICING (277bp)

E1 E3 = Exon SKIPPING (110bp)

Trans-spliced products

E1 DT-A = 1st EVENT, 196bp. *Trans*-SPLICING BETWEEN 5' ss OF TARGET & 3' ss OF PTM.

DT-A E3 = 2nd EVENT, 161bp. *Trans*-SPLICING BETWEEN 3' ss OF TARGET & 5' ss OF PTM.

FIG.8B

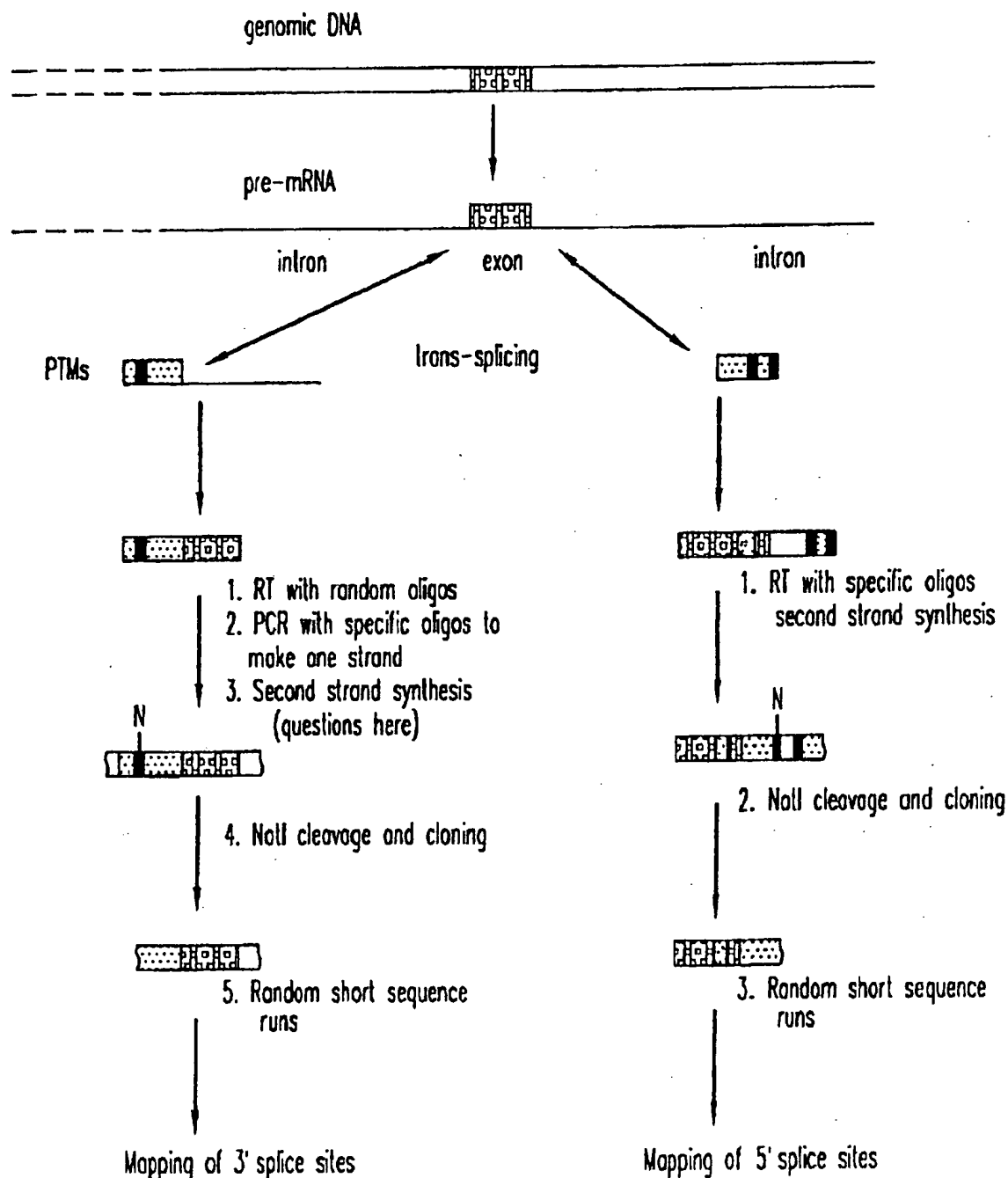
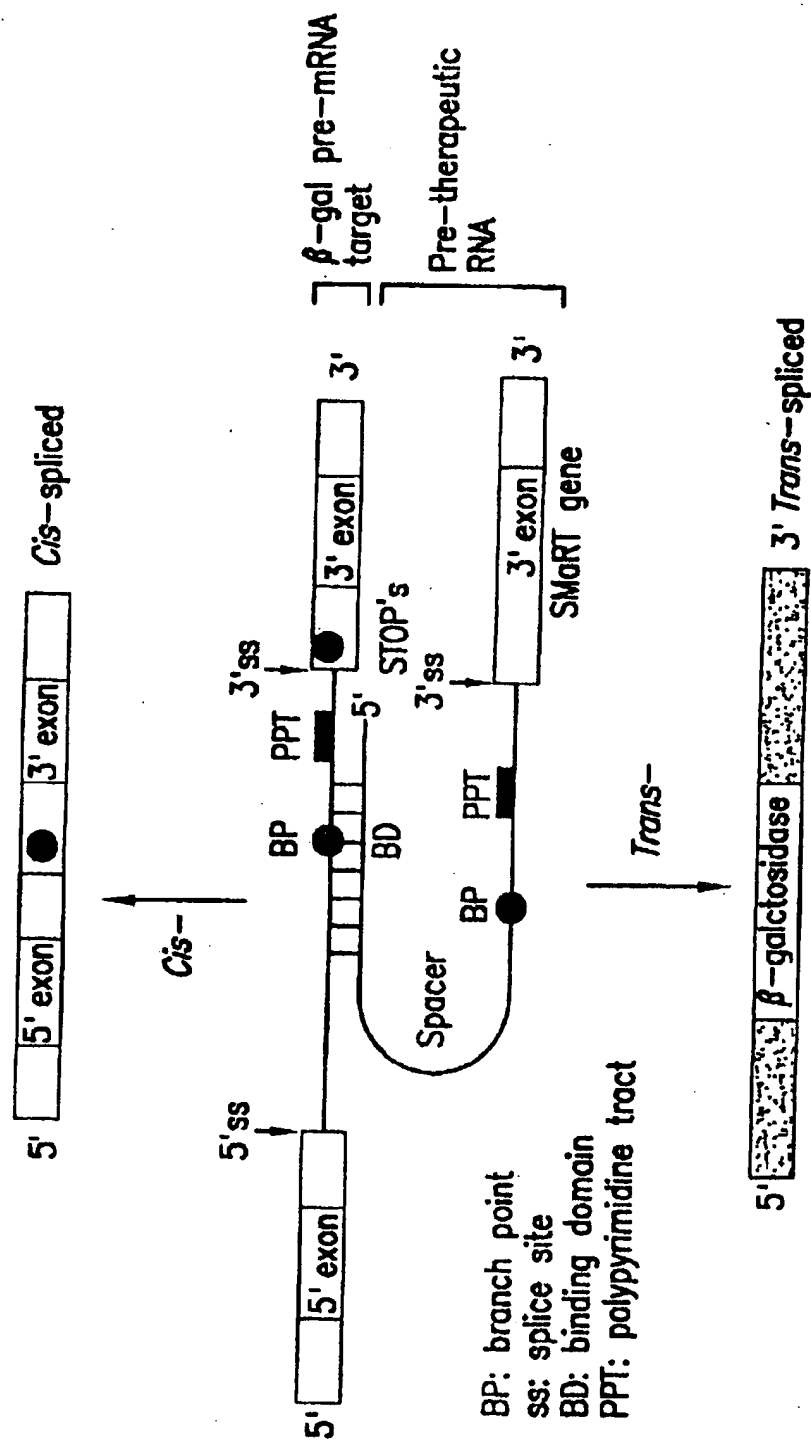


FIG.9







**FIG. 11A**

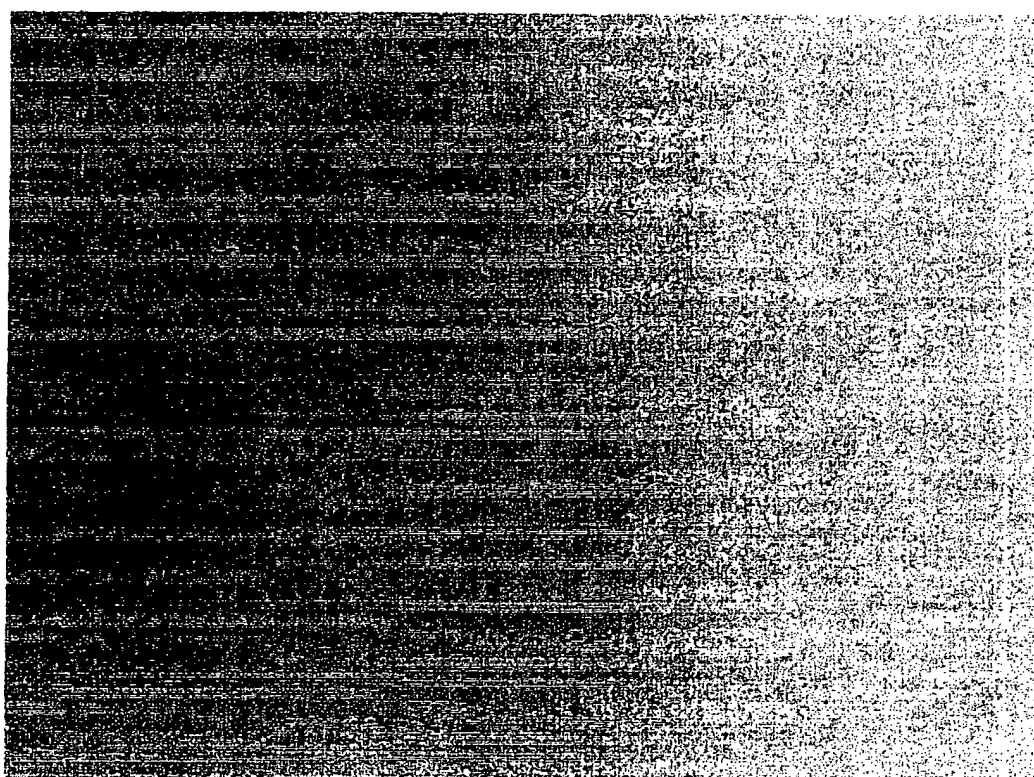
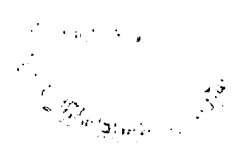


FIG. 11B



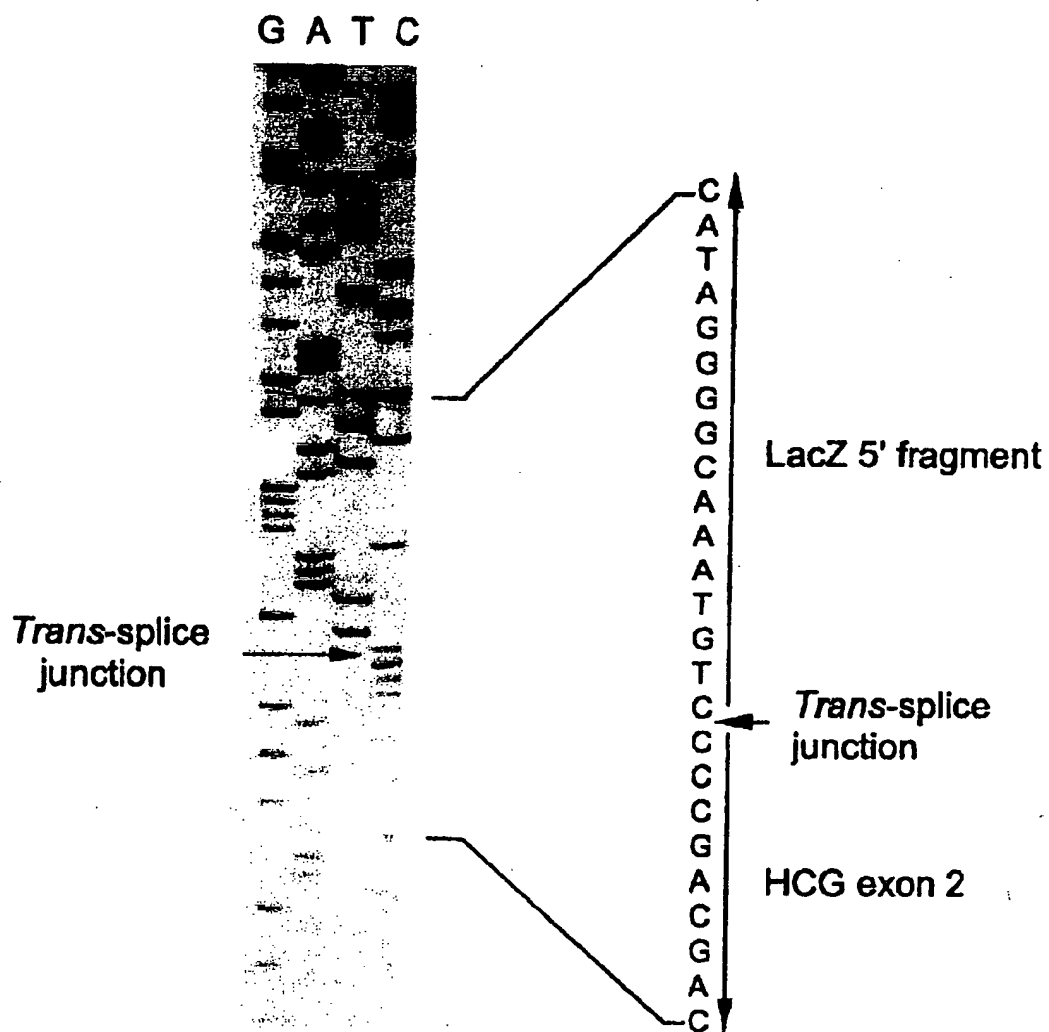


FIG.12A

**Biolac-TR1**

## Splice junction

GGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAACCGCAACCGTGGTCGGCTTACGGCGGTGATTT

## 2. NUCLEOTIDE SEQUENCES OF THE *trans*-SPLICED PRODUCT (195 bp)

**Bialac-TR1**

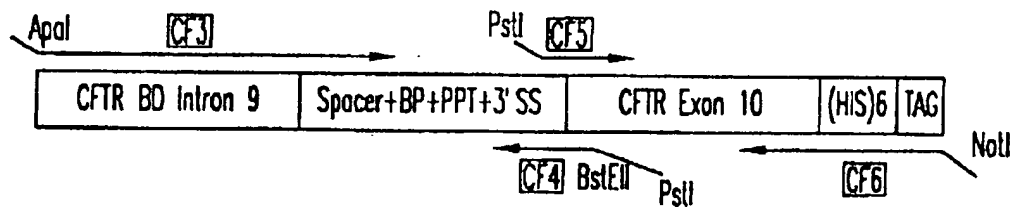
## Splice junction

HCGR2

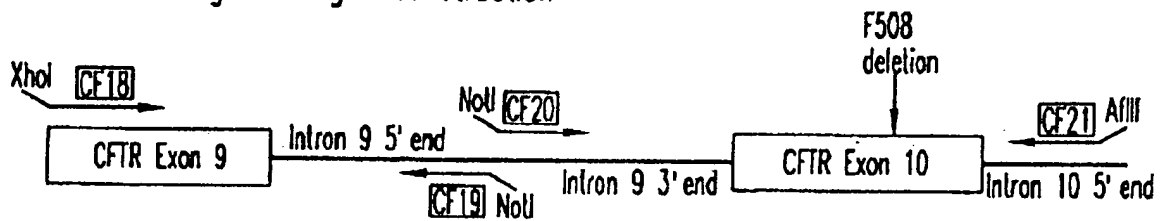
GAGCATGGCGGGACATGGGCATCCAAAGGAGCCACTTGGGCCADGGTGC GG  
TCCGZ

**FIG. 12B**

# CFTR Pre-therapeutic molecule (PTM or "bullet")



## CFTR mini-gene target-construction



## Trans-splicing Repair

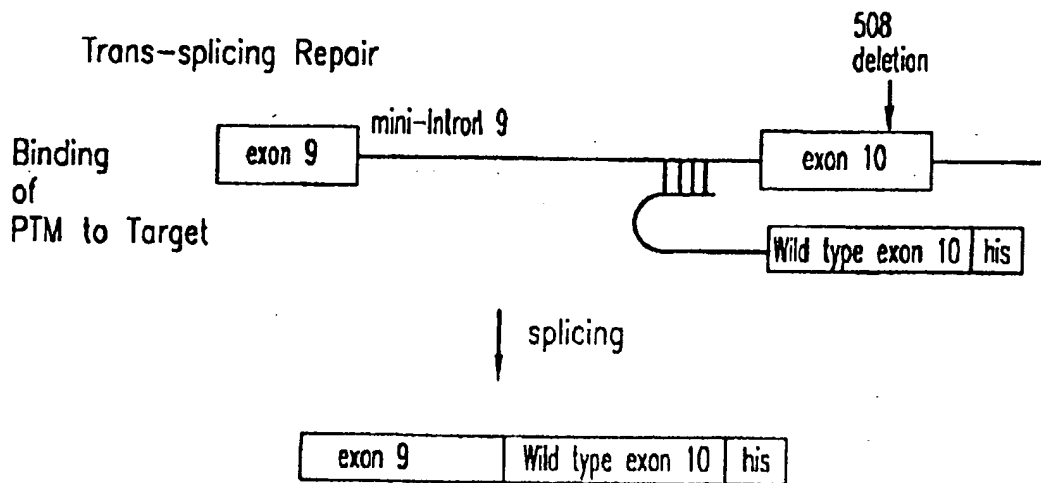


FIG.13

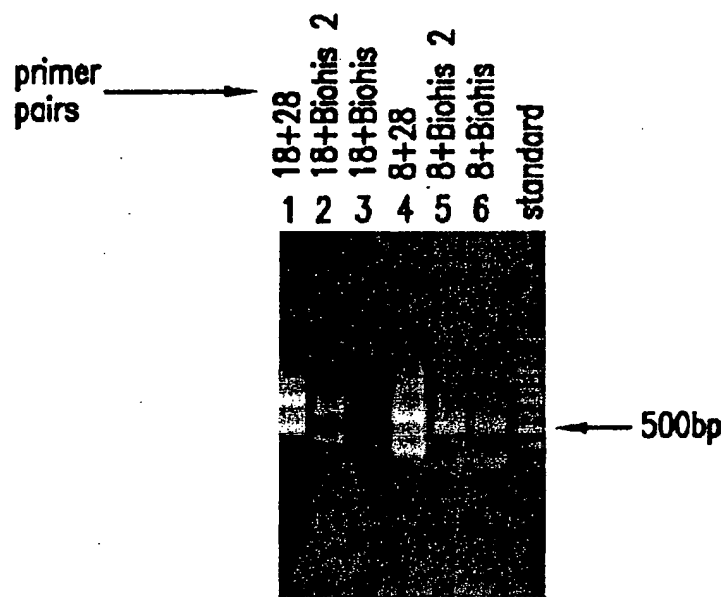


FIG.14

DNA sequence 500 b.p. GCTAGCGTTTAA ... TGCCACTCCAC linear

Positions of Restriction Endonucleases sites (unique sites underlined)

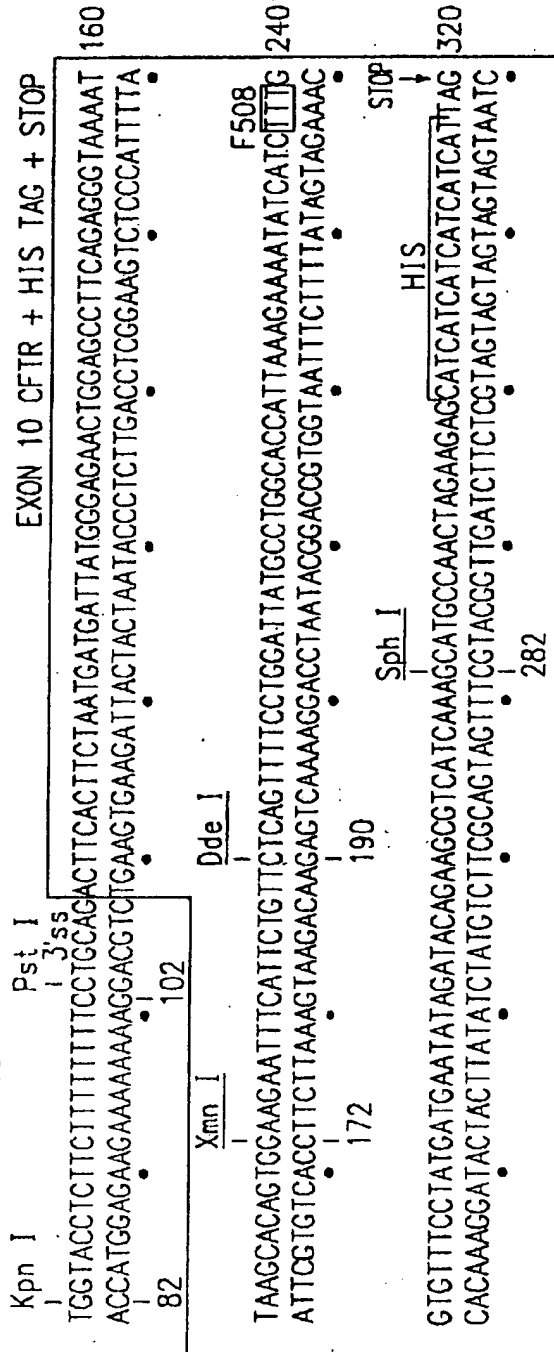
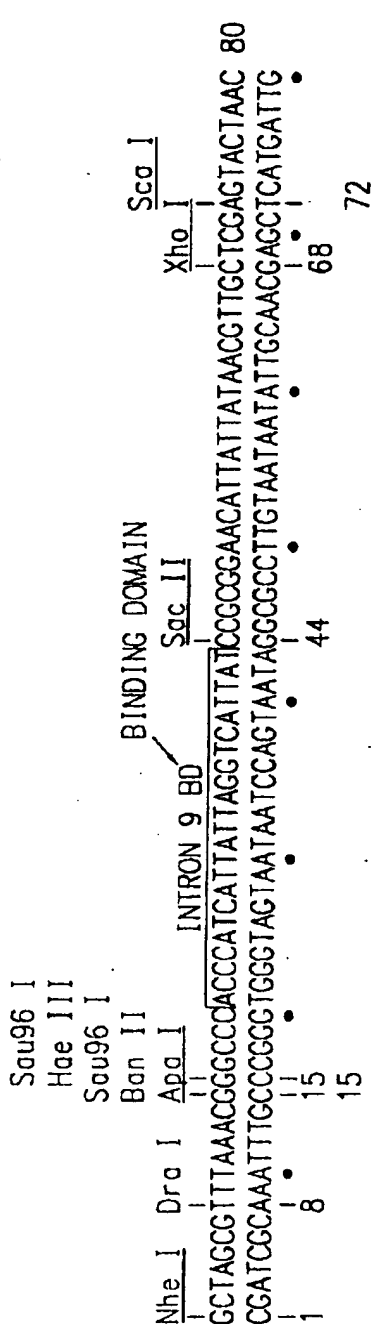


FIG.15A

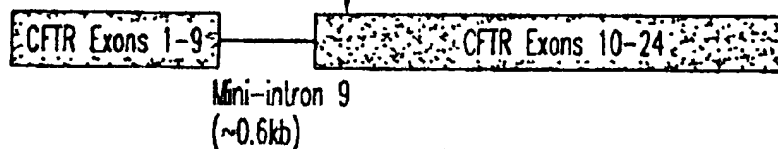






+

CFTR Target  
(mini-gene)



Cotransfect PTM and target molecules in HEK 293 cells  
and detect repaired CFTR mRNA by RT-PCR.

Repaired  
CFTR mRNA

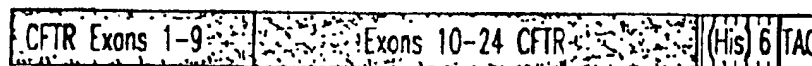


FIG.16

CFTR BD intron 9	Spacer+BP+PPT+3'SS	CFTR exon 10	Spacer+BP+PPT+5'SS	CFTR BD intron 10
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Double Splicing  
PTM

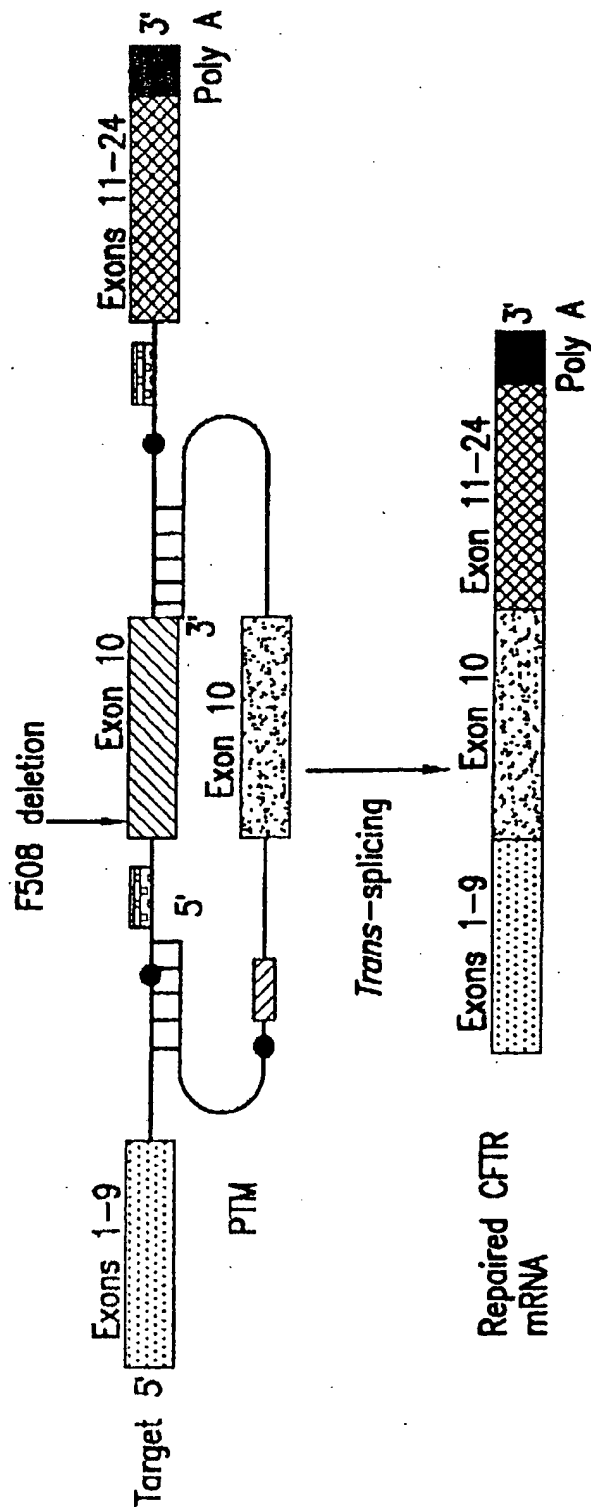


FIG.17

DOUBLE TRANS-SPLICING SPECIFIC TARGET

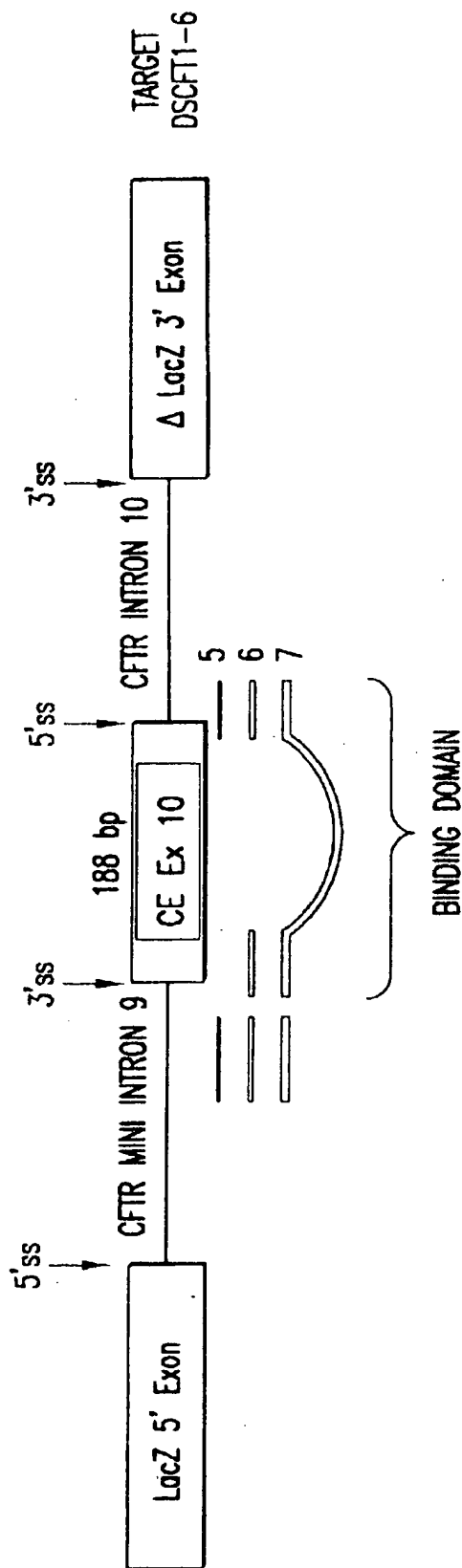


FIG.18

# DOUBLE TRANS-SPLICING PTMs

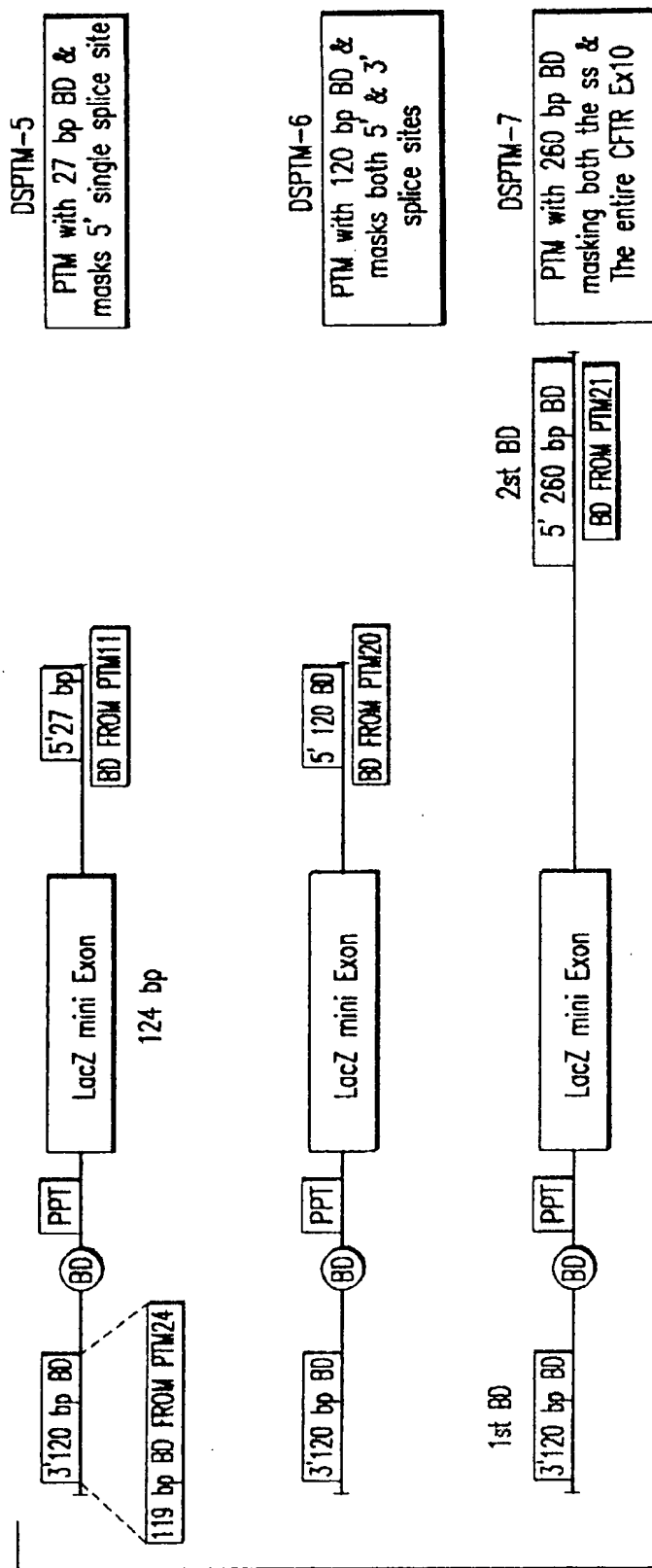
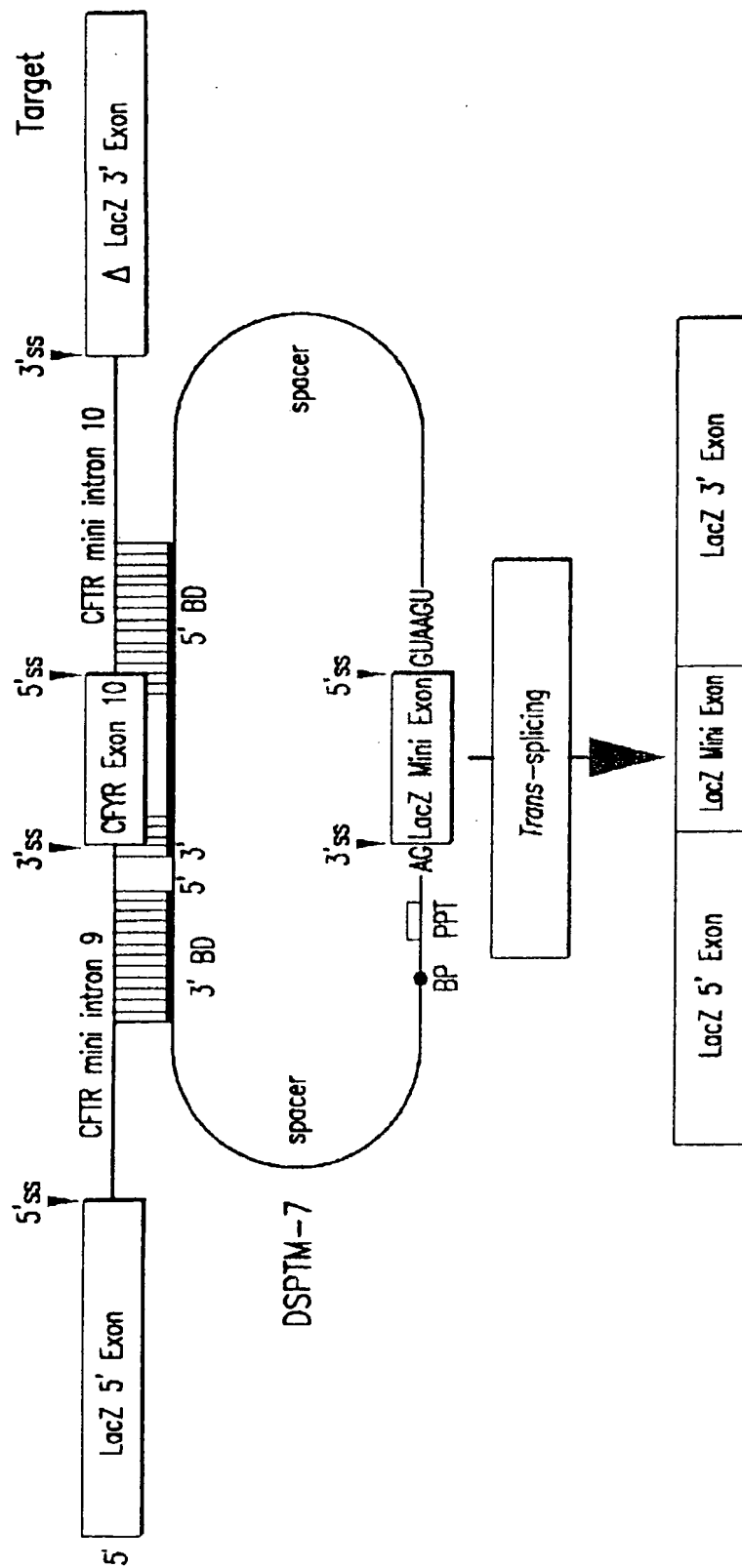


FIG.19

DOUBLE  
SPlicing  
PTMs

# DOUBLE TRANS-SPLICING $\beta$ -GAL MODEL

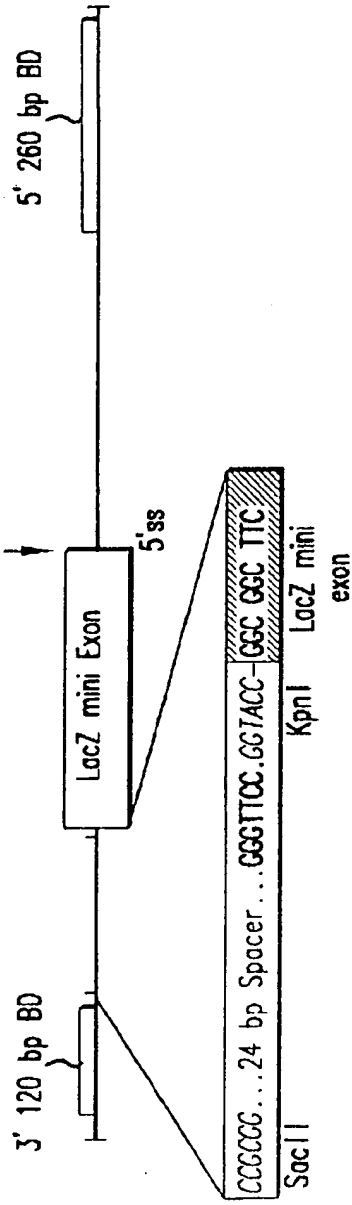


Repaired LacZ mRNA

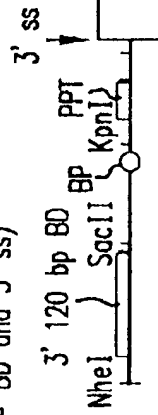
FIG.20



DSPTM8: ( $\Delta$  3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



PTM29 (lacks 2nd BD and 5' ss)



PTM30 (lacks 1st BD and 3' ss)



FIG.22

Mutants



2020-05-06

# ACCURACY OF DOUBLE TRANS-SPlicing REACTION

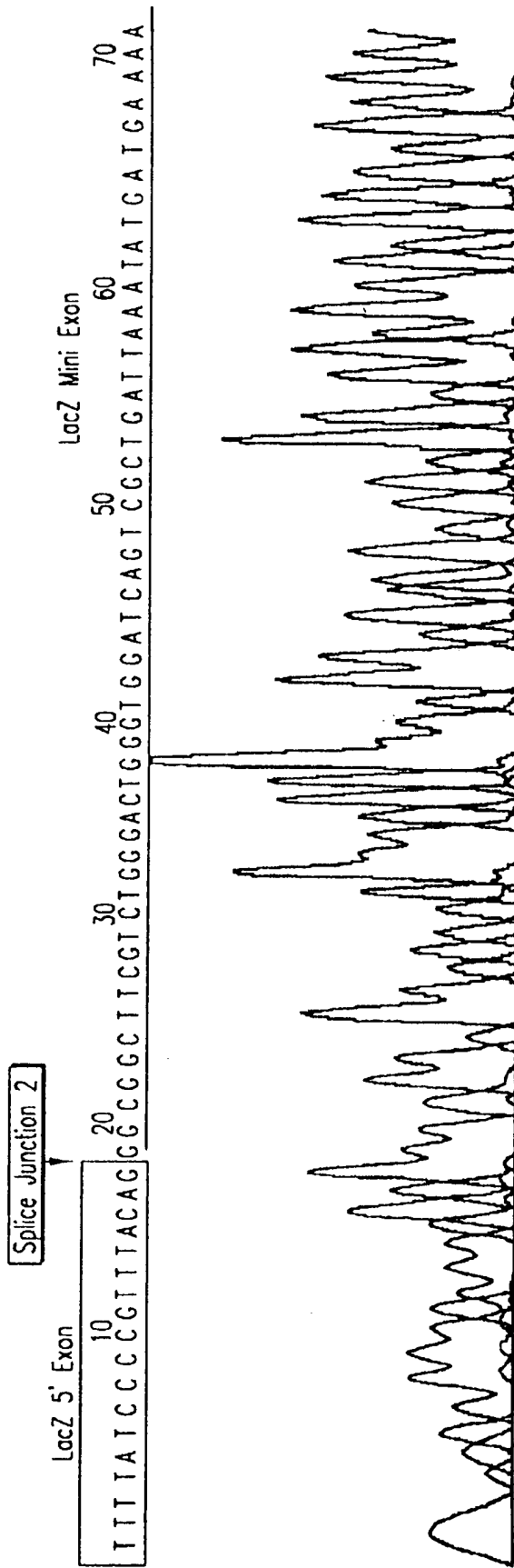


FIG.23A

# ACCURACY OF DOUBLE TRANS-SPICING REACTION

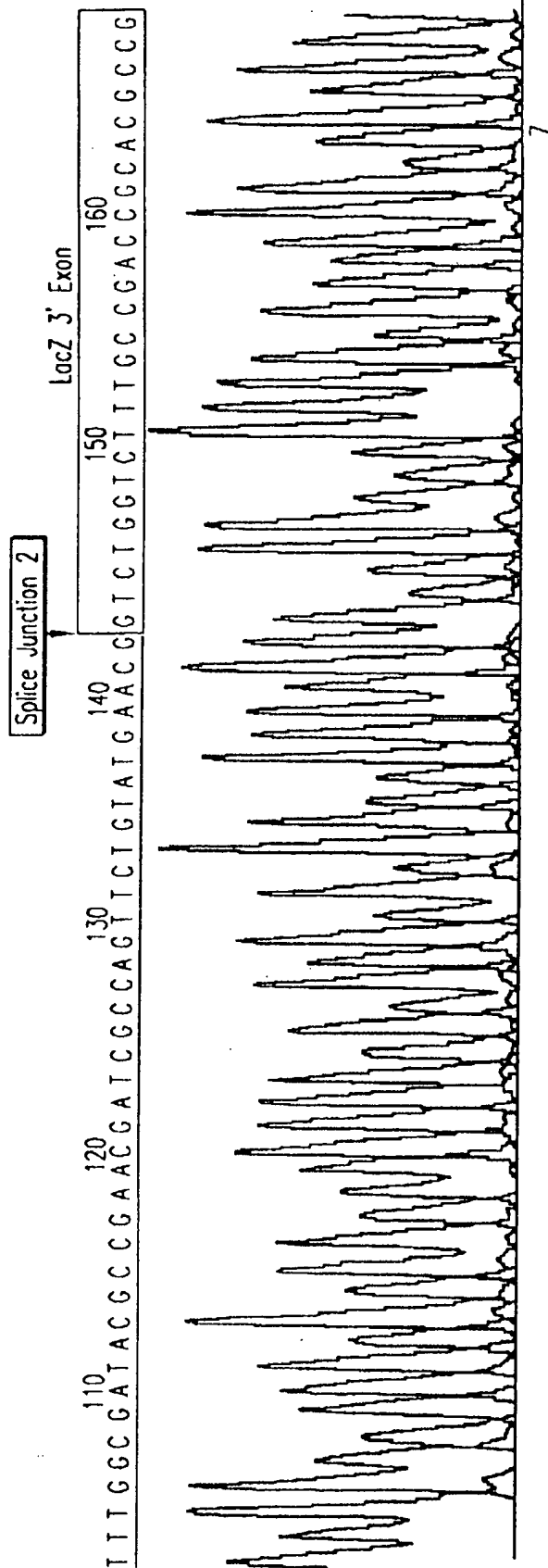


FIG.23B

# Double Trans-splicing Produces Full-length Protein

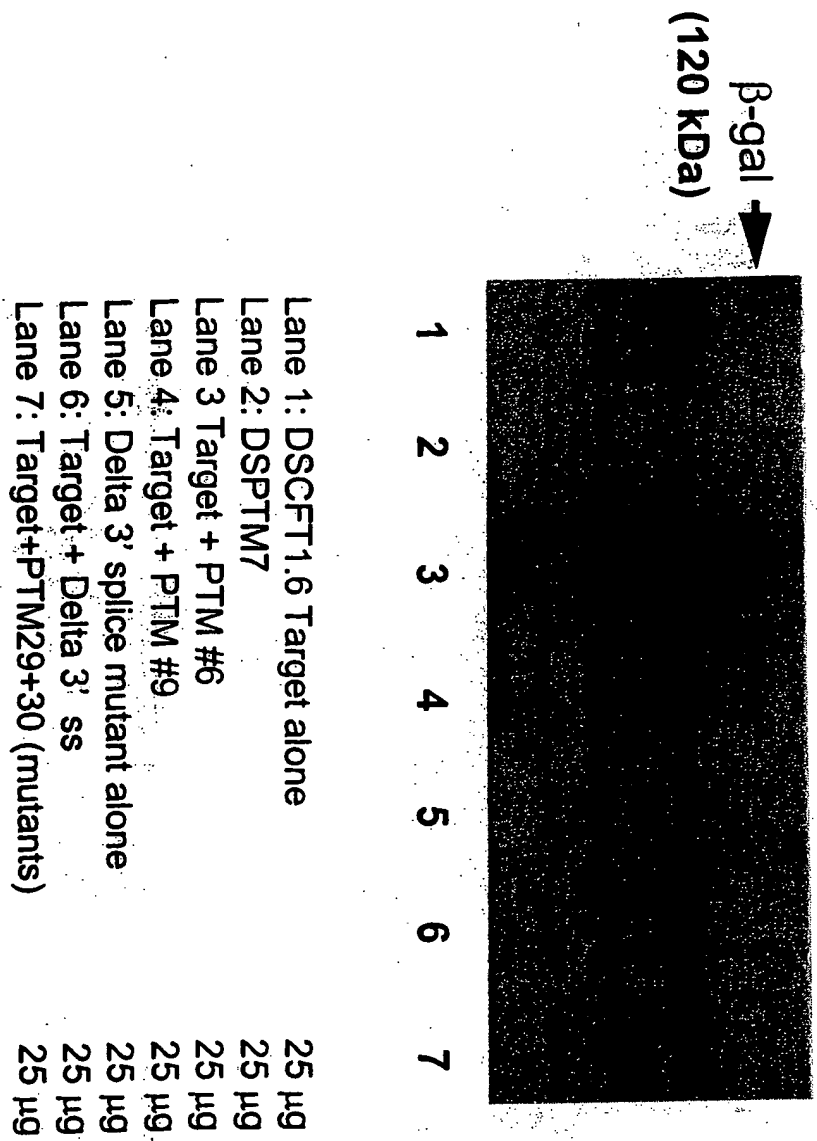
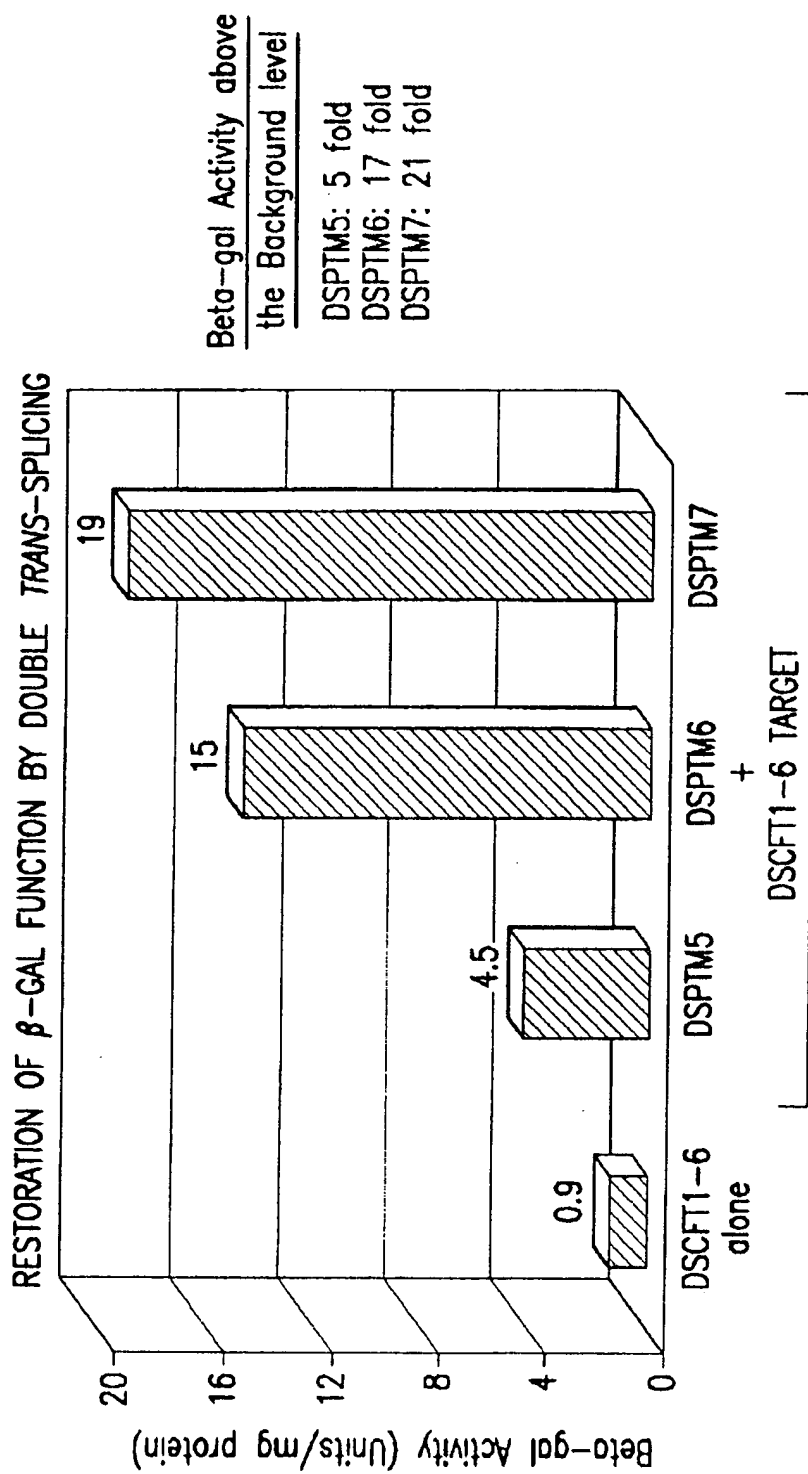
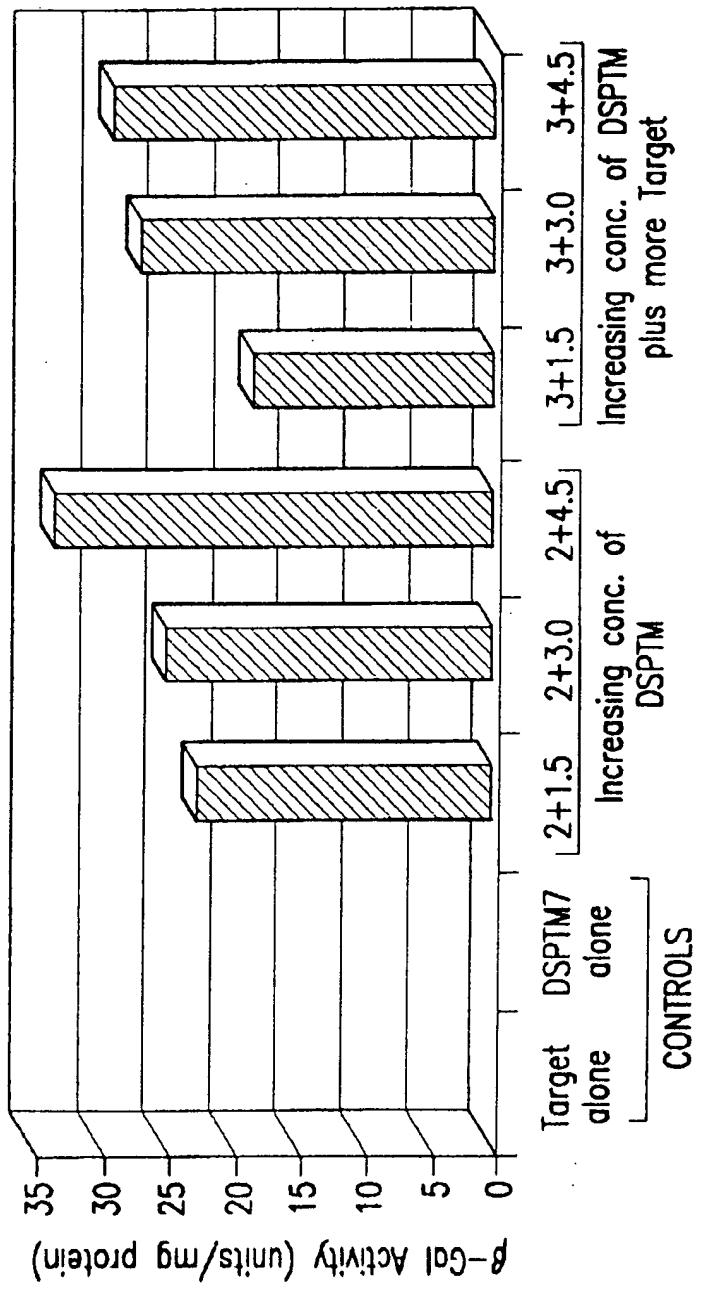


Figure 24





DOUBLE TRANS-SPLICING: TITRATION OF TARGET & PTM



The current level of beta-gal activity due to double *trans*-splicing is ~ 1-1.5% of the best single splice model (3' exon replacement)

FIG.27

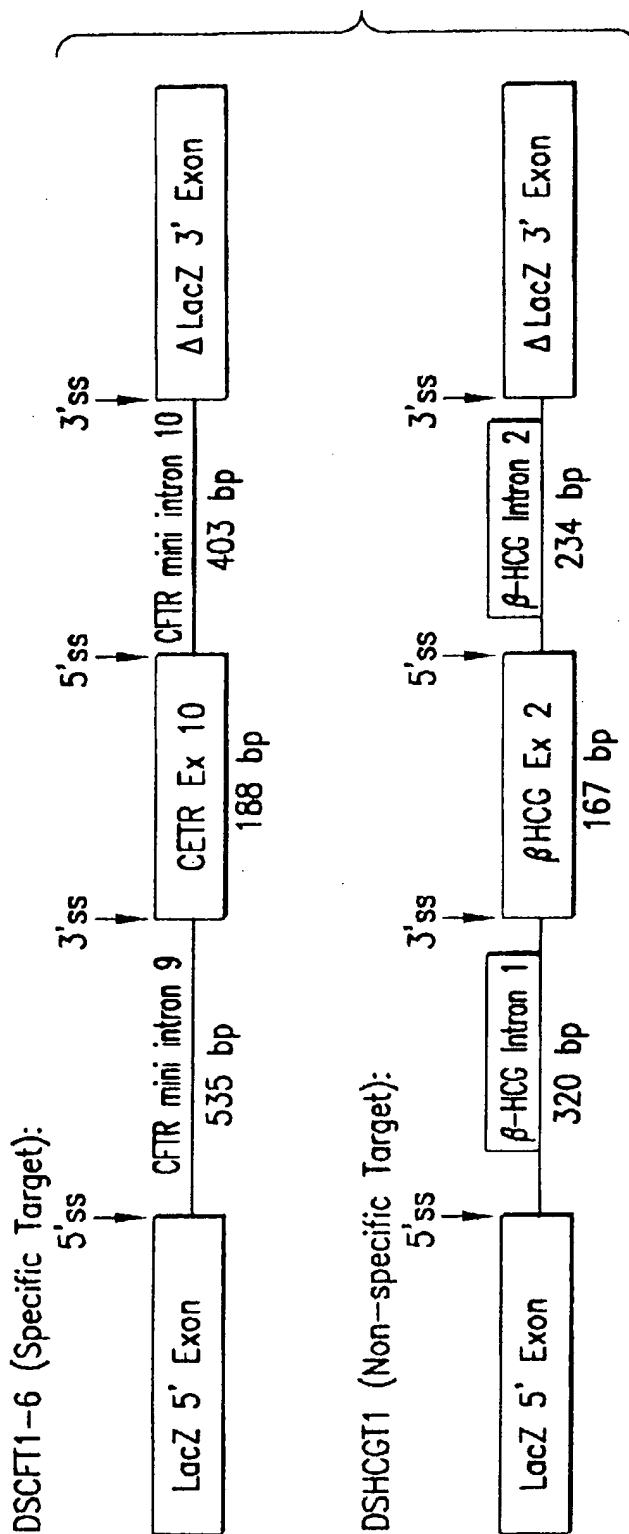
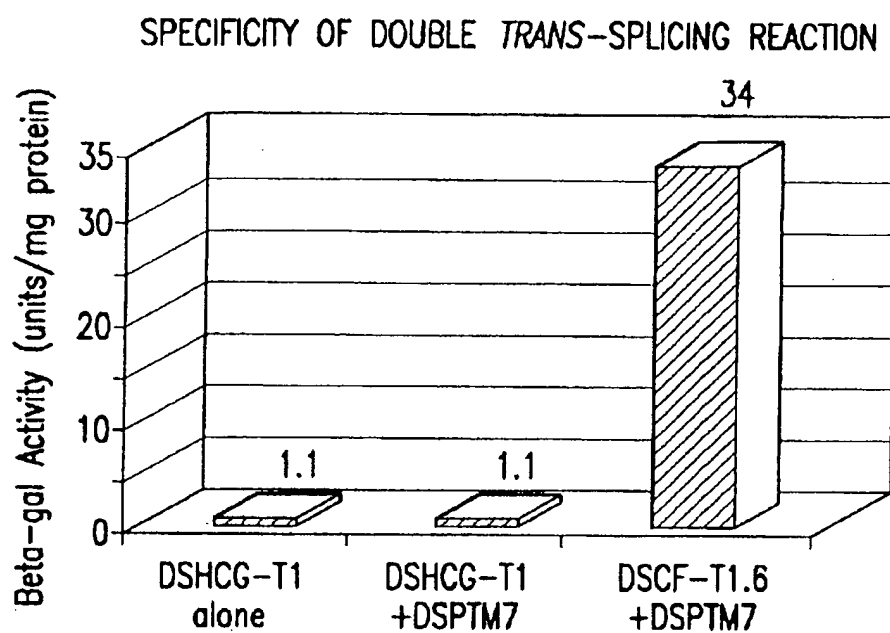


FIG.28





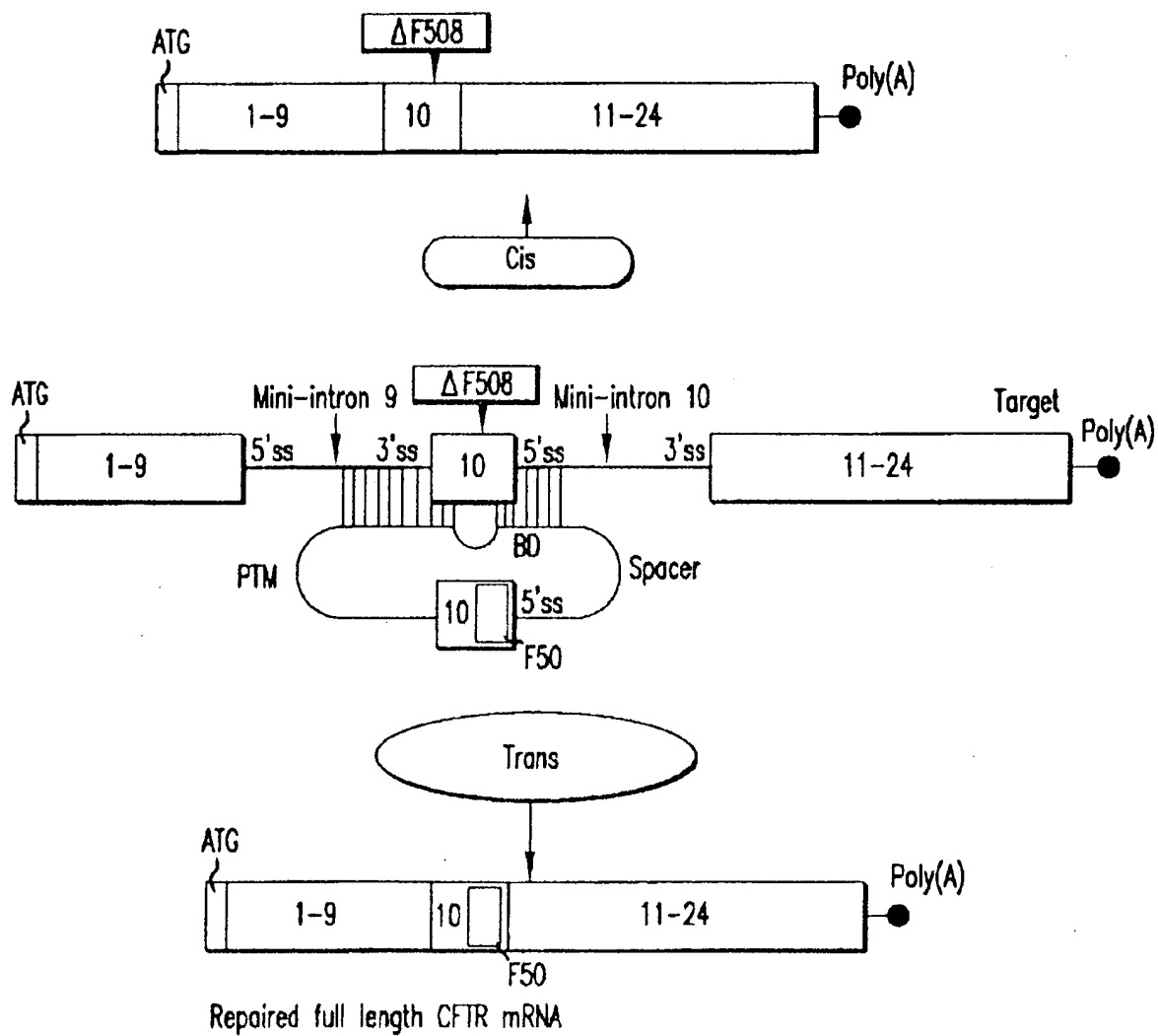
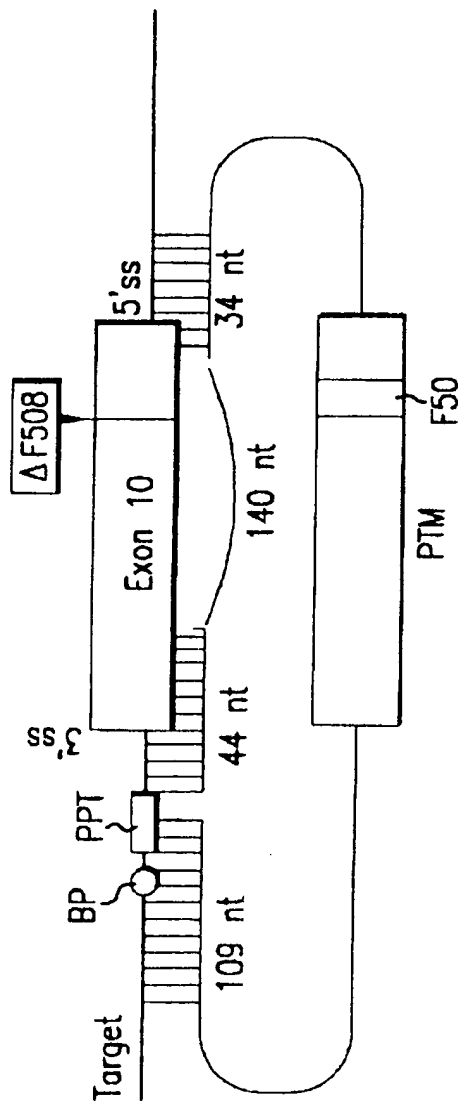


FIG.30

PTM with a long binding domain masking  
two splice sites and part of exon 10  
in a mini-gene target



ACGAGCTTGCATCATGATGATGCGGAGTTAGACCAAGTGAAGGCAAGATCAACATTCGG  
GCGCATCAGCTTTTCAGGCCAATTCAGTGGATCATGCCCGGTACCATCAAGGAGAAATAT  
C7TCGCGGTGAGTACGACGAGTACCGCTATCGCTGGTGAATTAAGGCCGTGTCAGTGGAGGAG

MCU in exon 10 of PTM

88 OF 192 (46%) bases in PTM exon 10 are not complementary to  
its binding domain (bold and underlined).

FIG.31

Sequence of a double  
*Trans*-spliced product

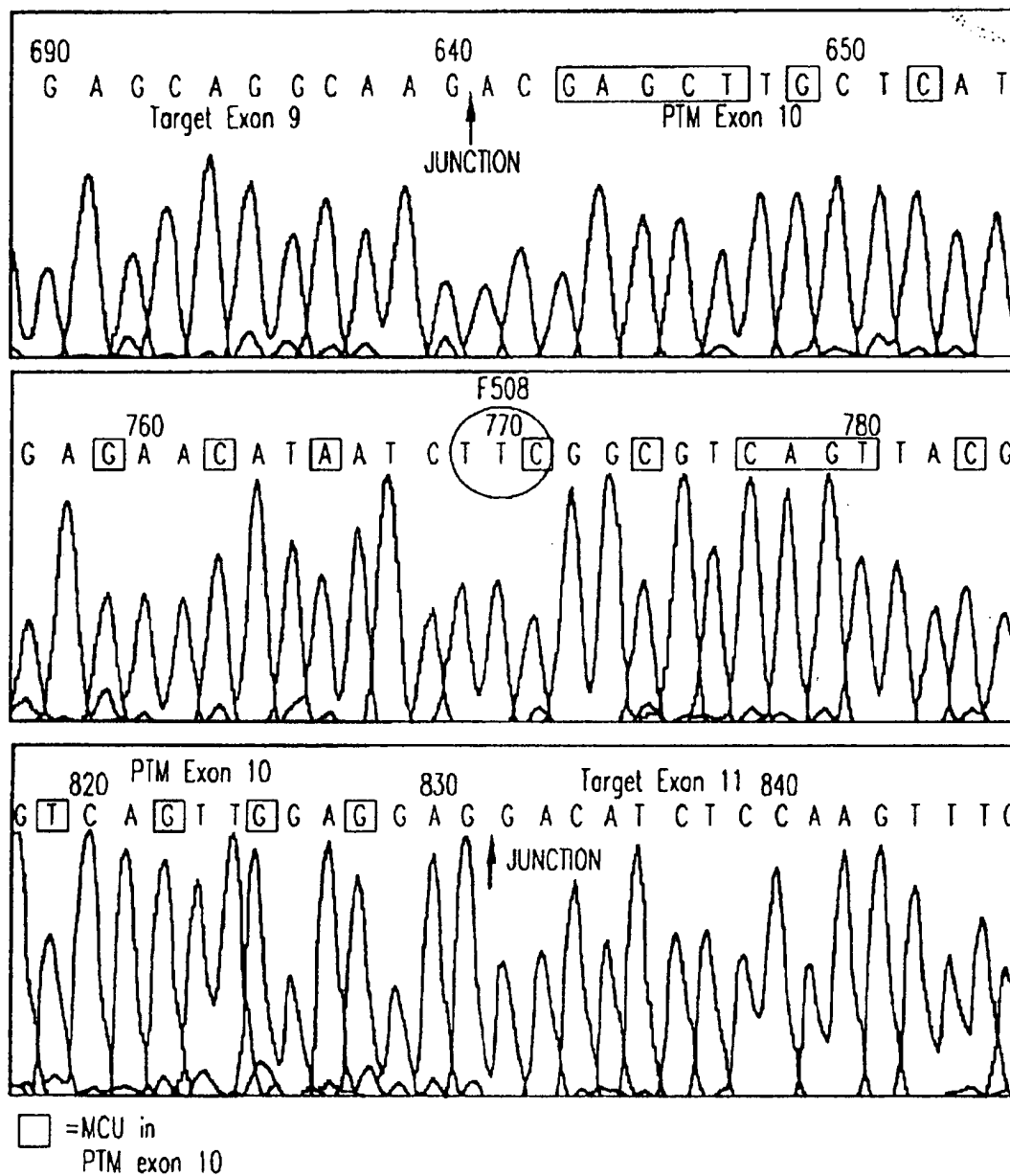


FIG.32

CF-TR Repair: 5' Exon-Replacement schematic diagram of a PTM binding to the splice site of intron 10 of a mini-gene target

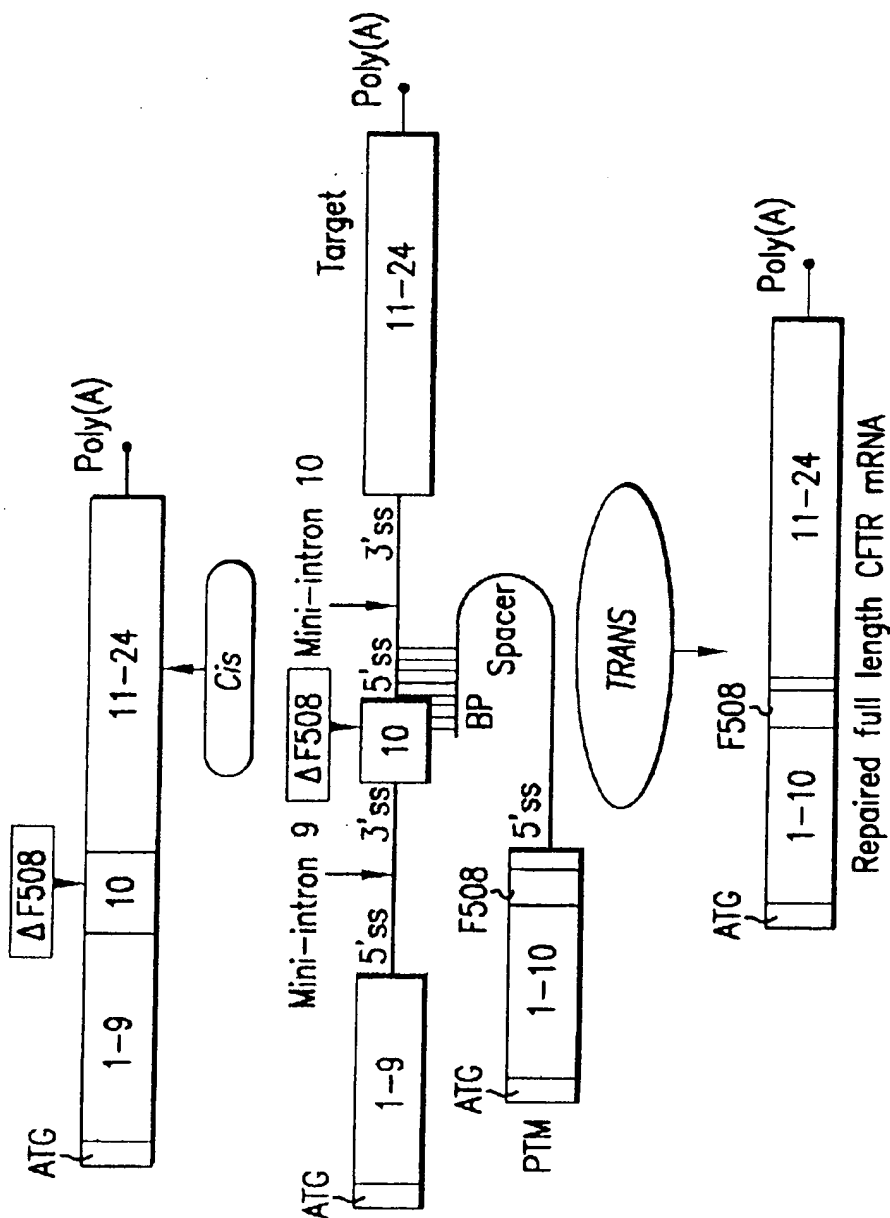


FIG.33

PTM with a short binding domain masking a single splice site in a mini-gene target.

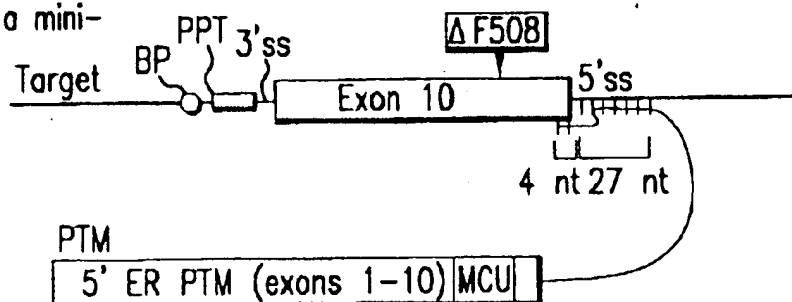


FIG.34A

PTM with a long binding domain masking two splice sites in a mini-gene target.

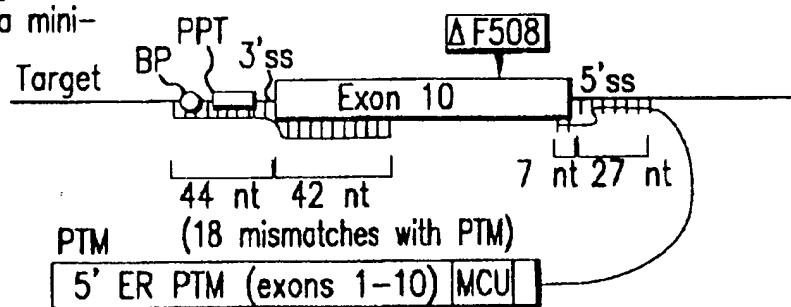


FIG.34B

PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

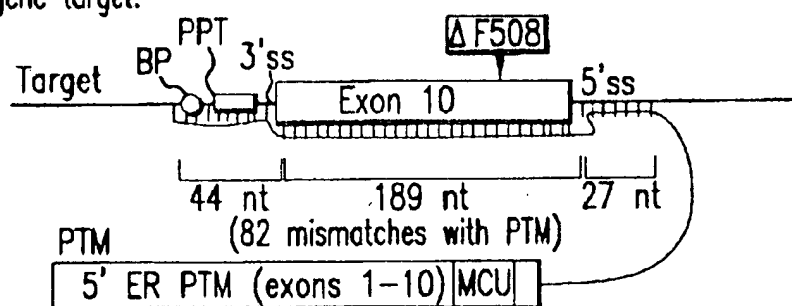
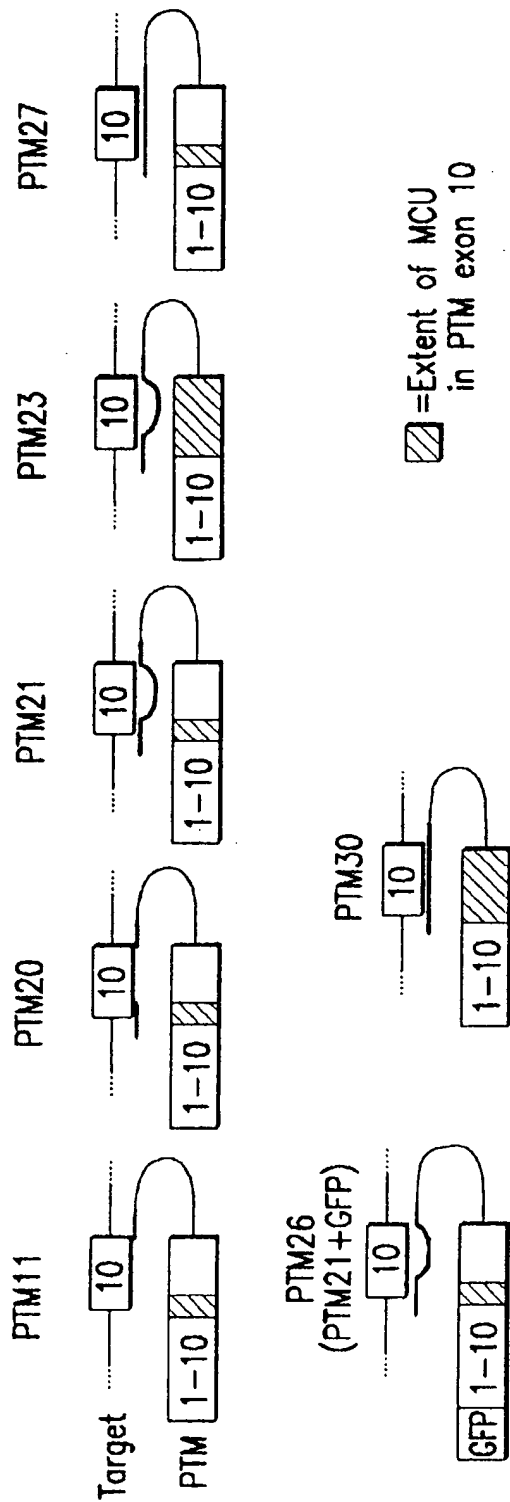


FIG.34C

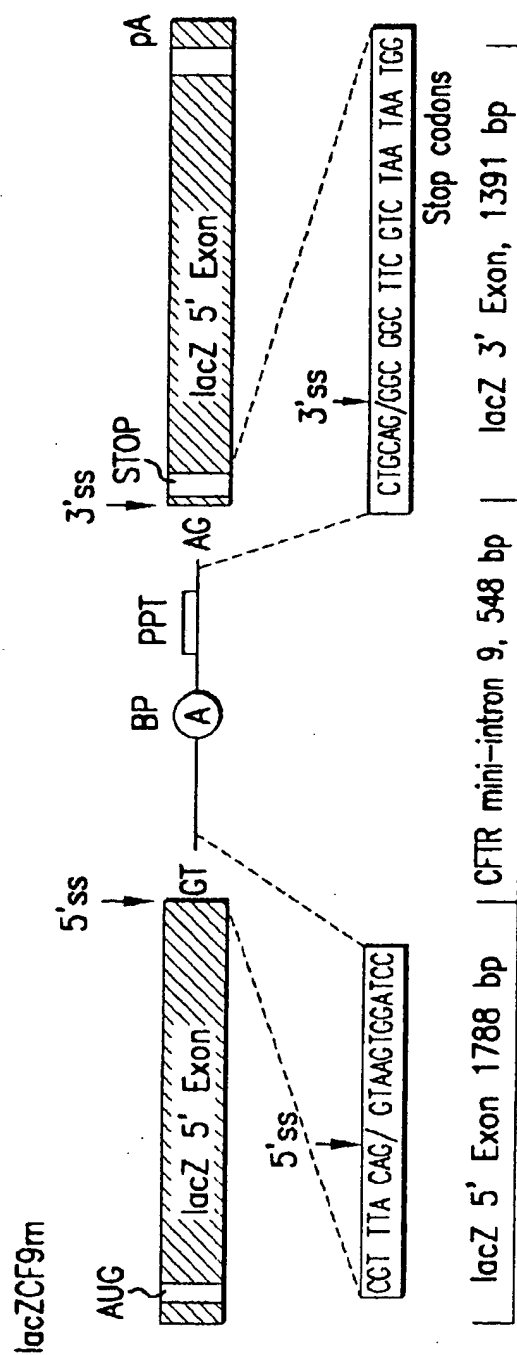


MCU in exon 10 of PTM  
88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain.

ACGAGCTTGCATCATGATGATGCGGAGTTAGACCAAGTGAAGCAAGATCAAACATTCCG  
GCGCATCAGCTTTTCAGCCAAATTCAGTTGGATCATGCCCGGTACCATCAAGGAGAACATAAT  
CTTCGCGGTCAGTTACGACGAGTACCGCTATCCGTCGTTAAGGCCGTGTCAGTTGGAGGAG

FIG. 35







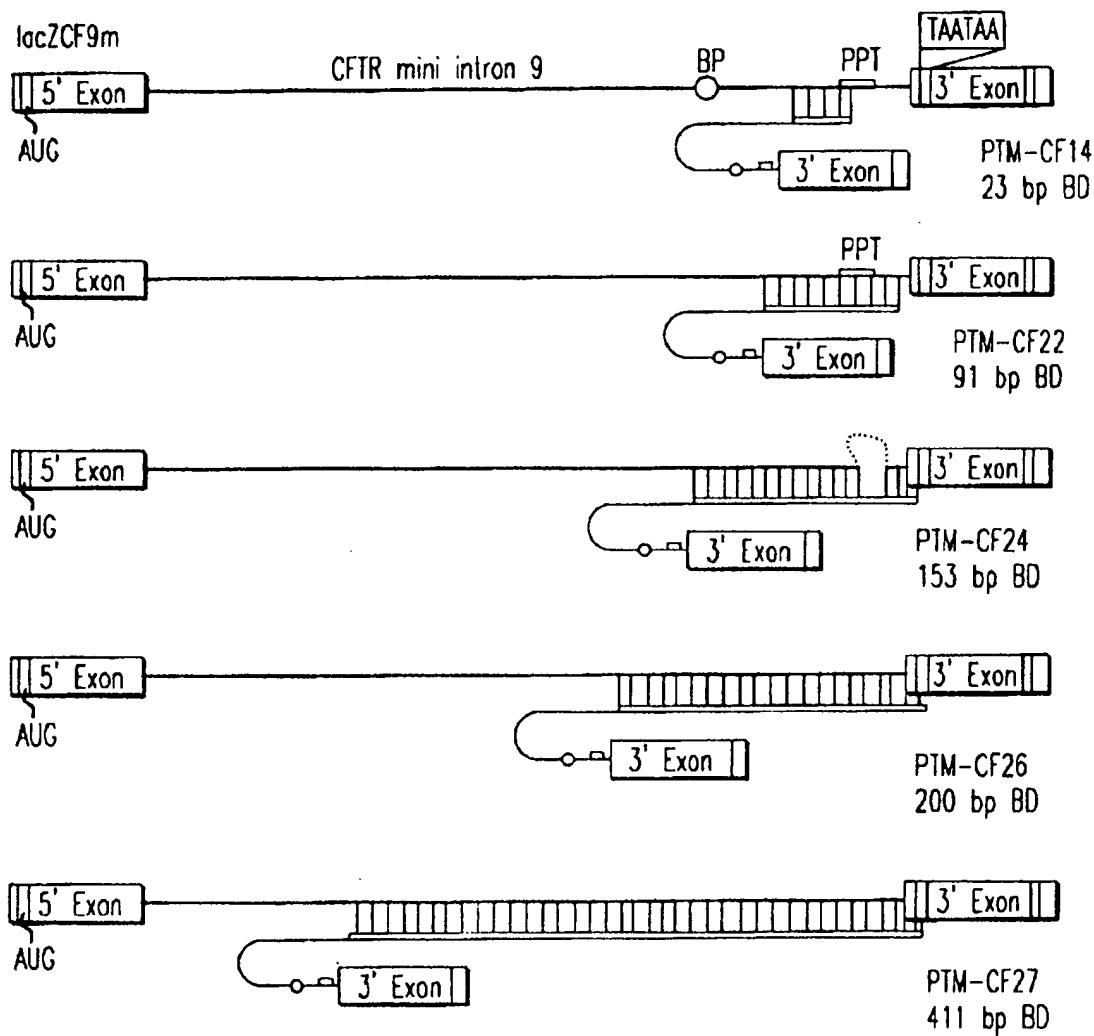
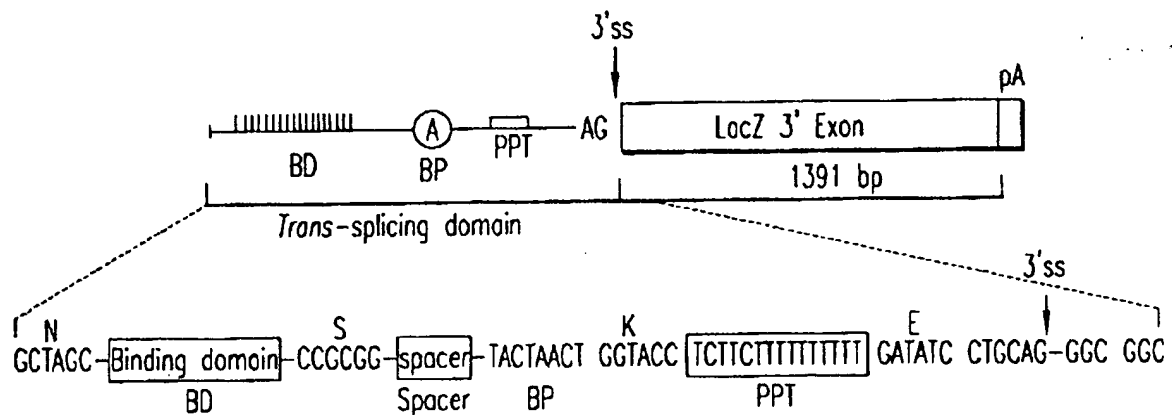


FIG.37B

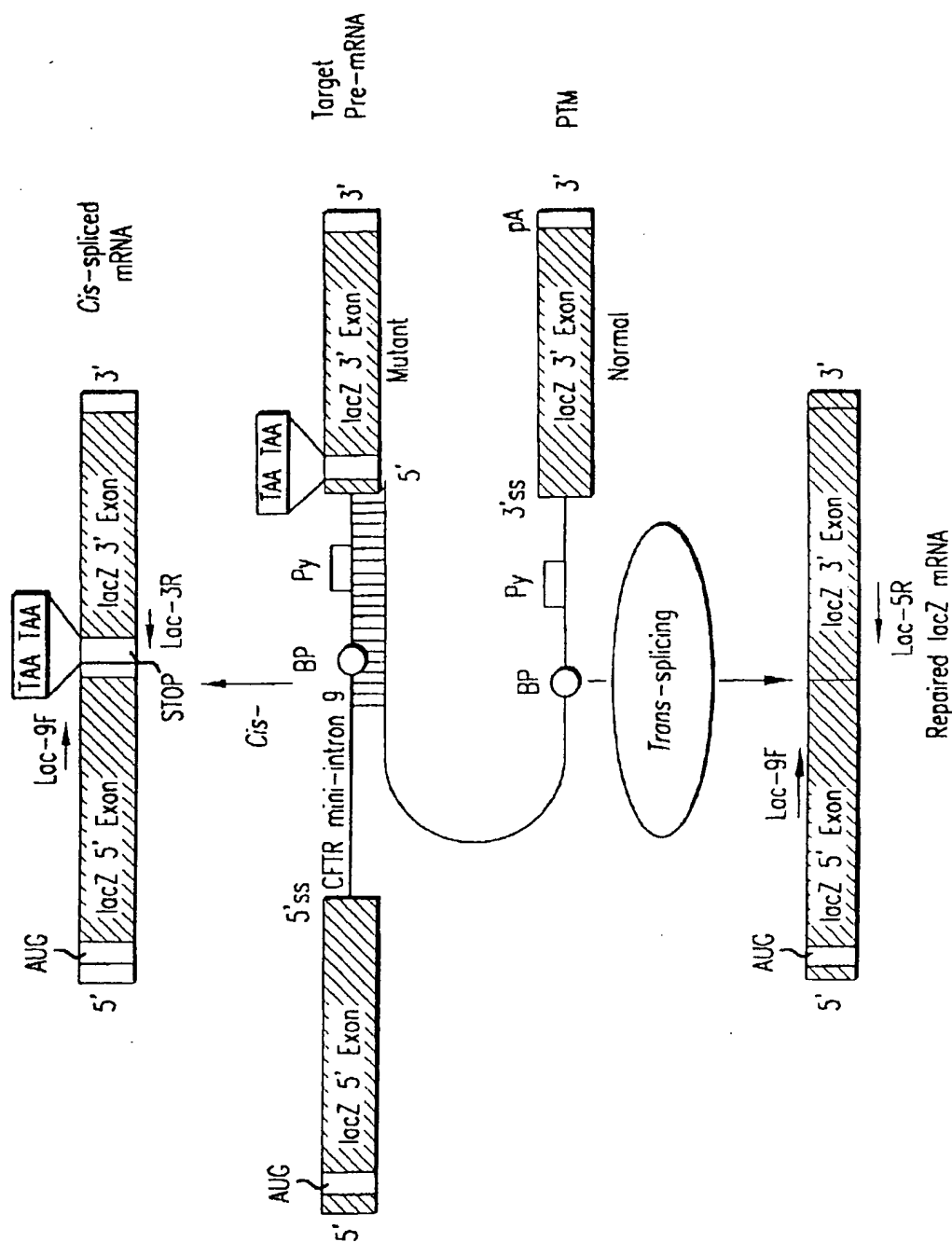


FIG.37C

2007070300000000

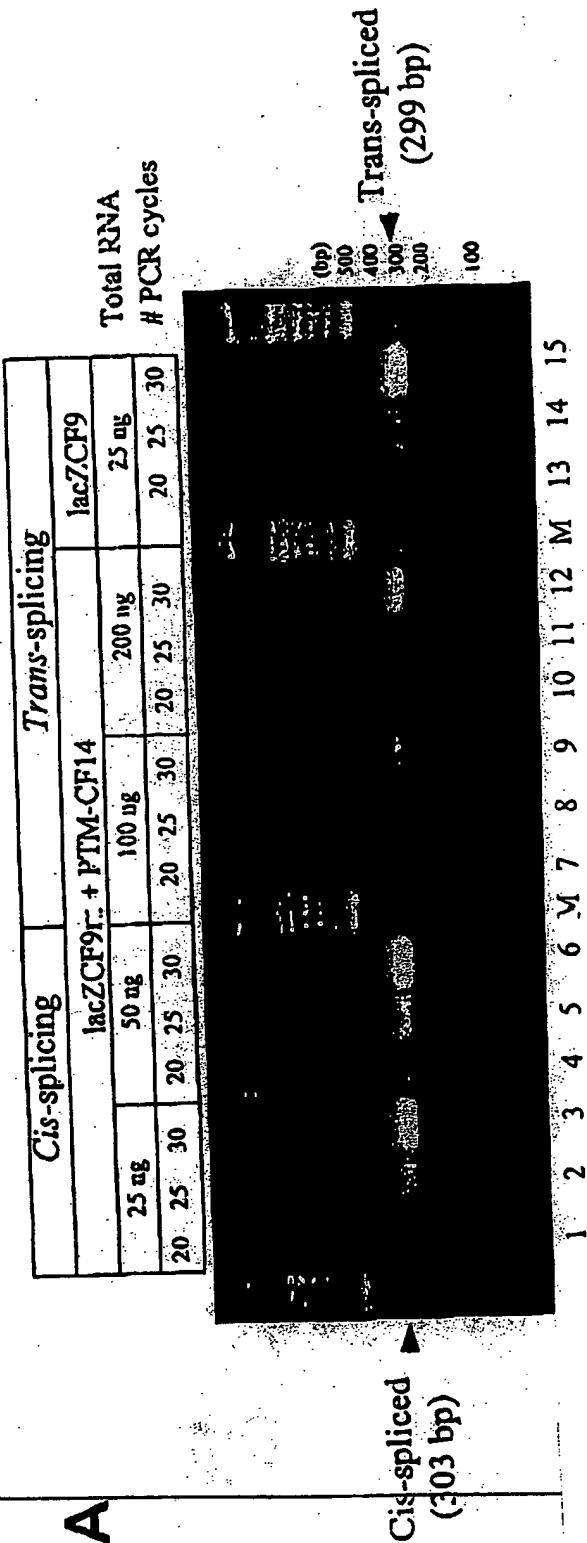
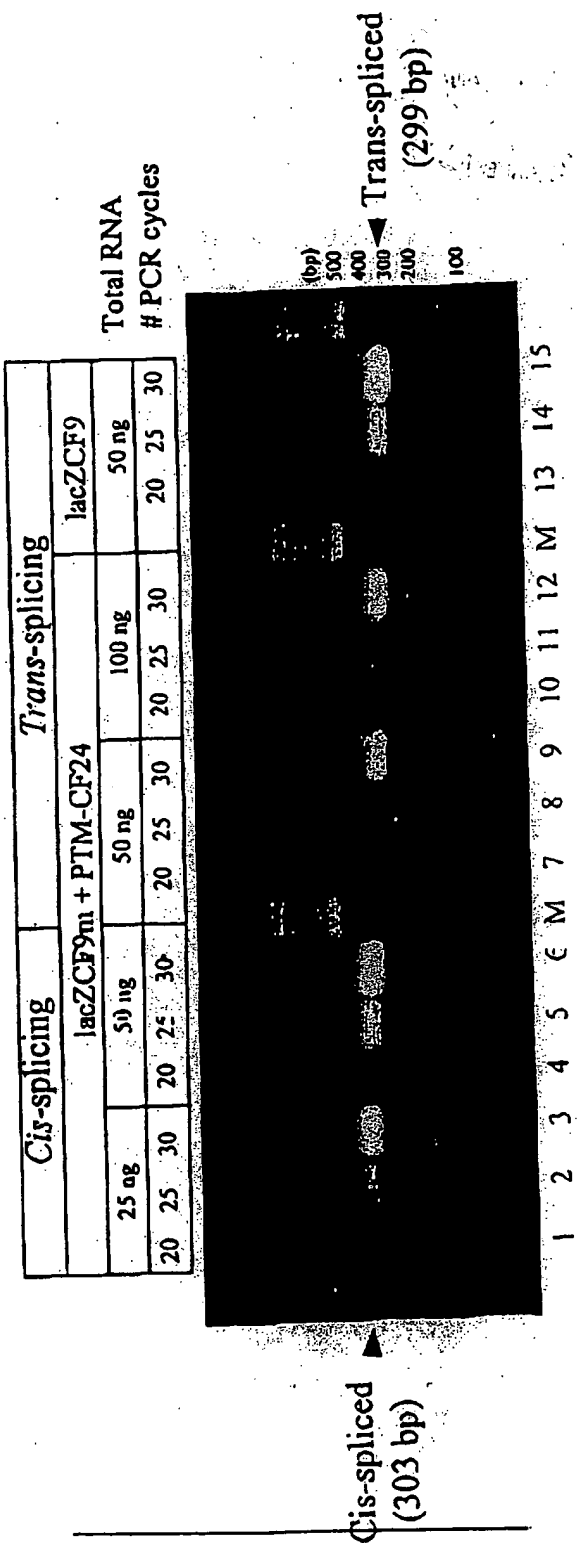


Figure 38A



B

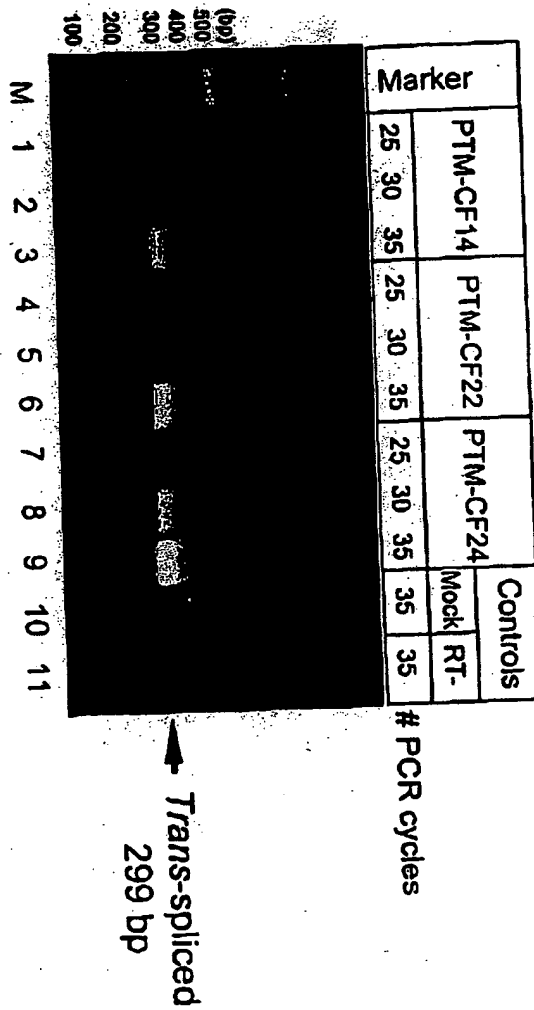


Figure 38B

01 040401 09

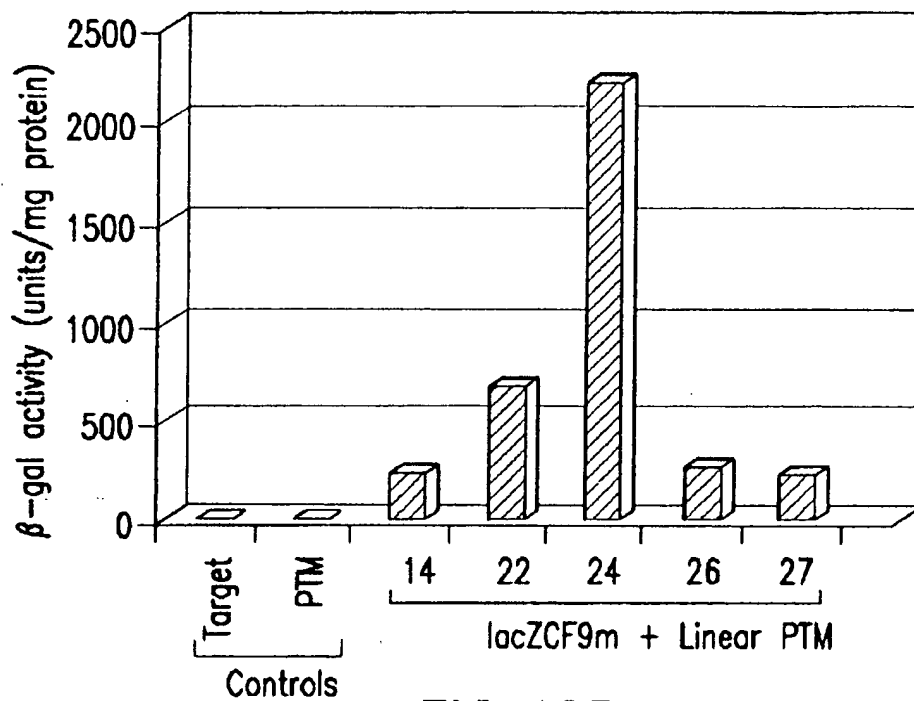


FIG.40B

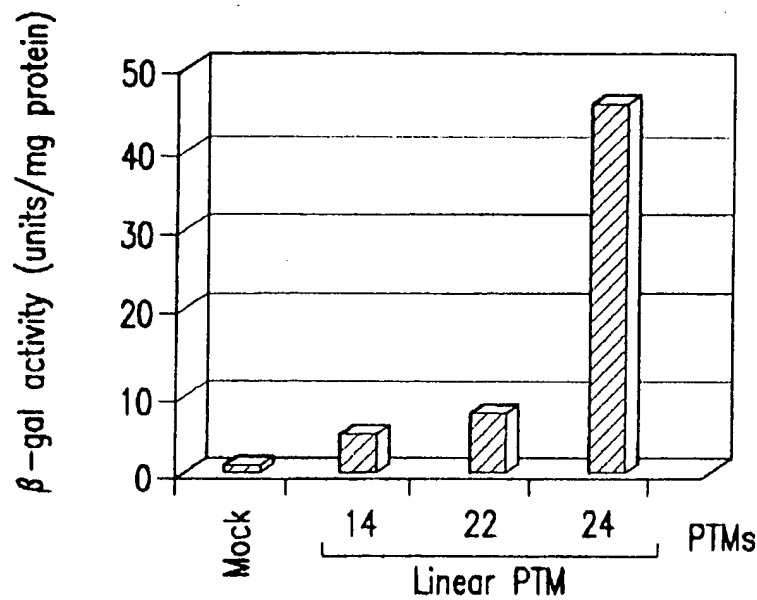


FIG.40C

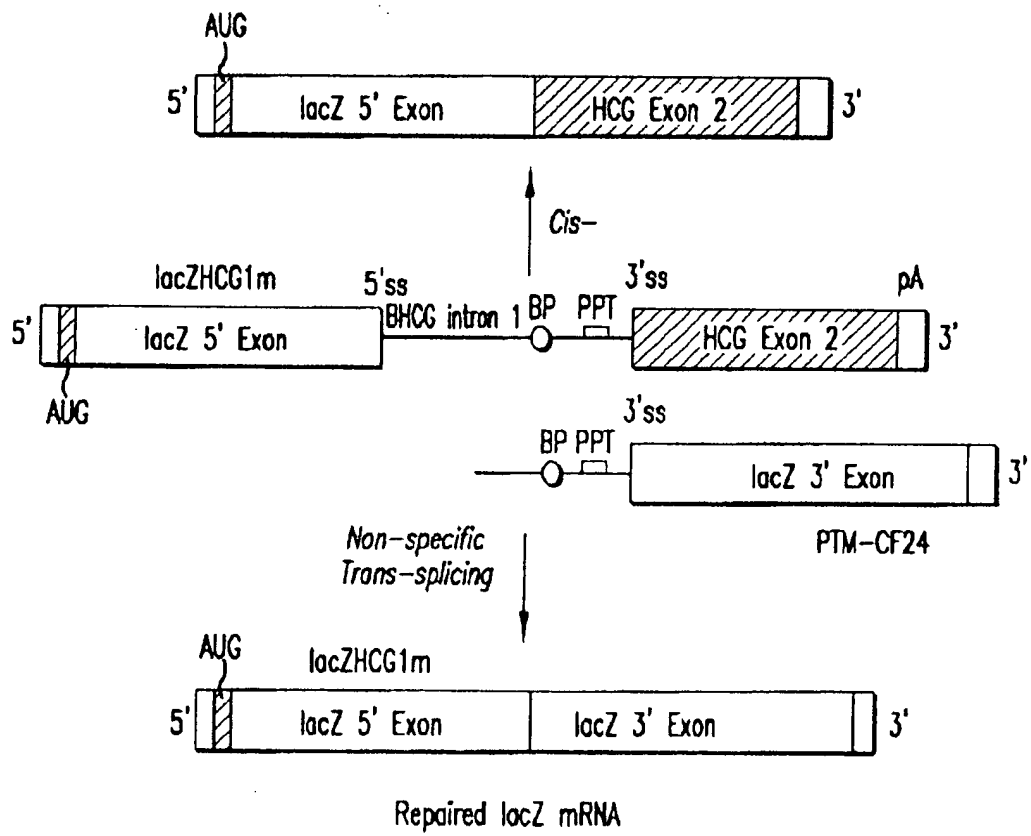


FIG.41A

100

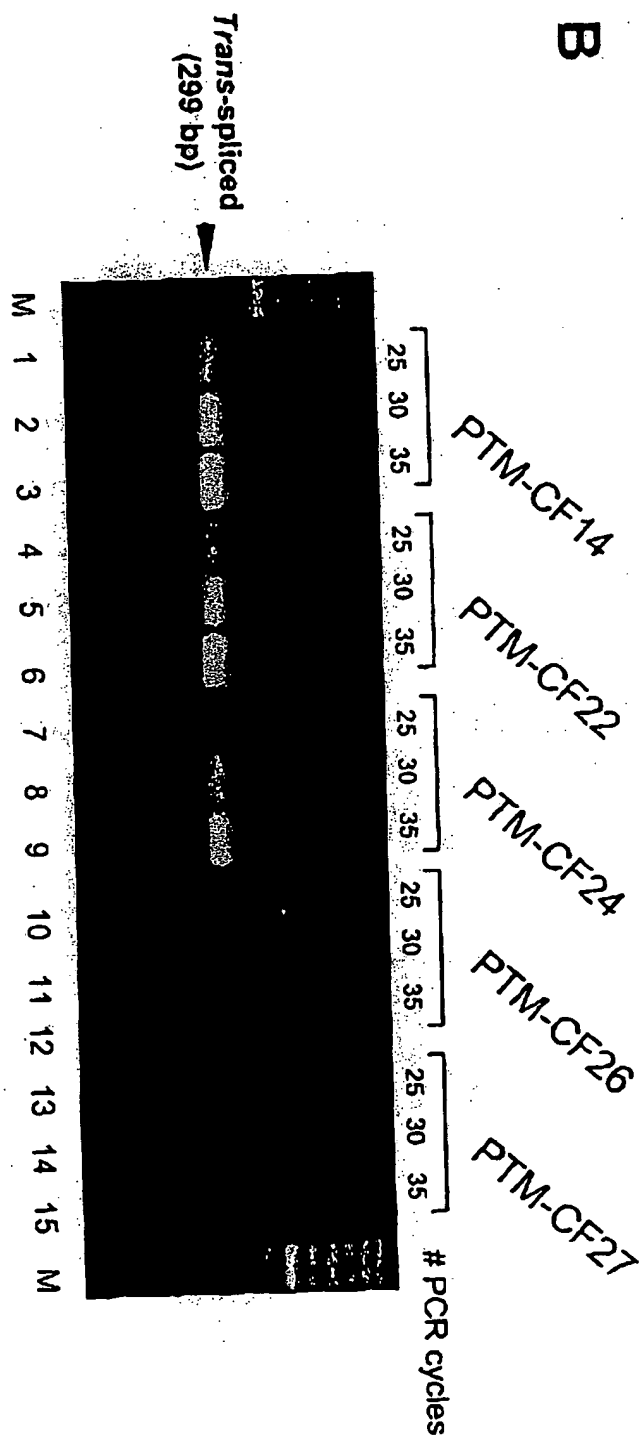
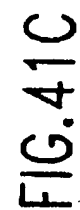


Figure: 41B

[illegible]





[illegible]

**153 bp BD underlined**

TTC TTATT TGTAA GATTCTA TTA ACTCAT TTGATT CAAAATATTT AAAATAC TTTCC TGTTCACCTACTCTGCTATGC

AC-CCGCG

FIG. 43A

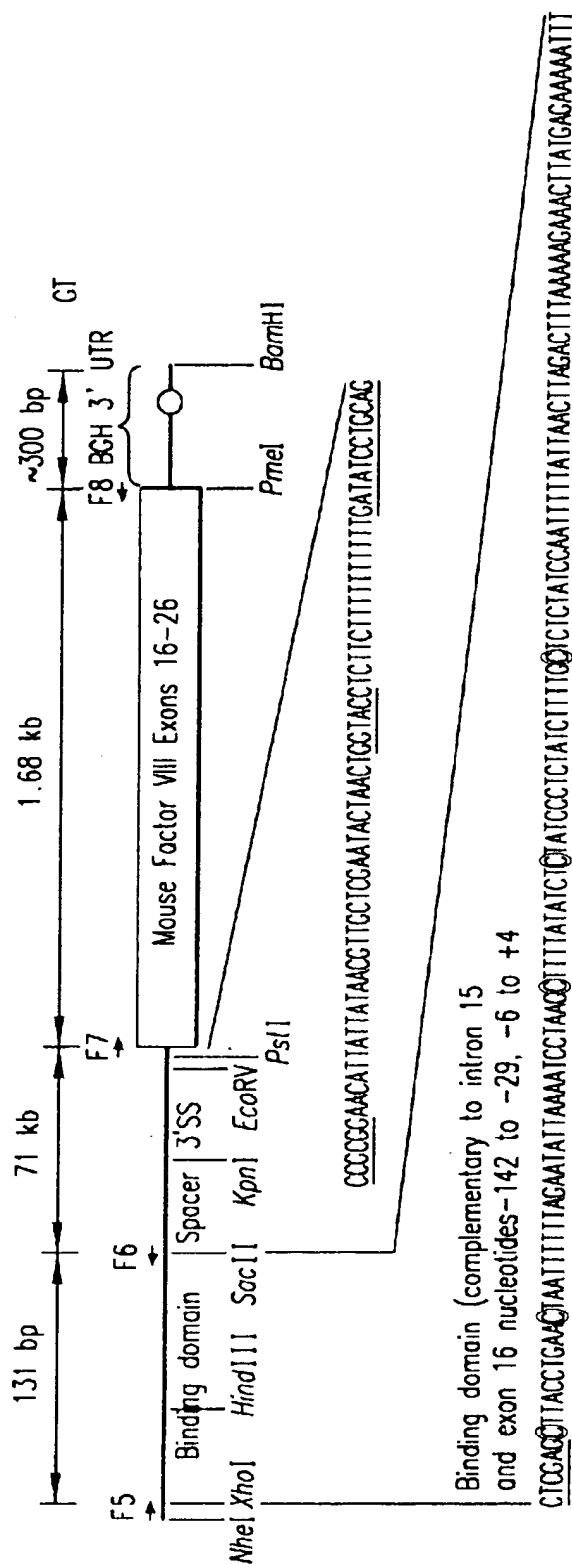
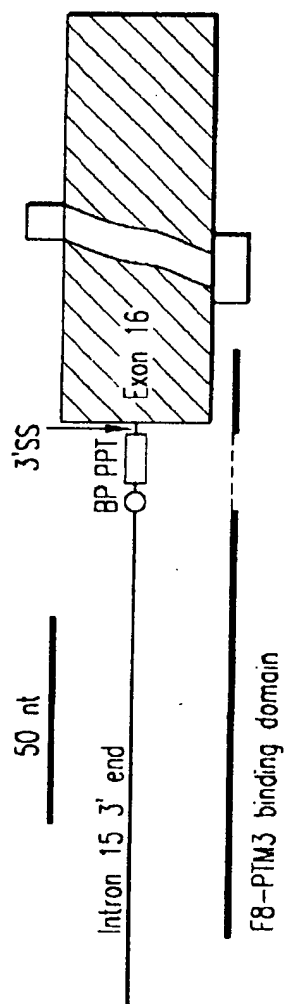


FIG. 44A

FIG.44B





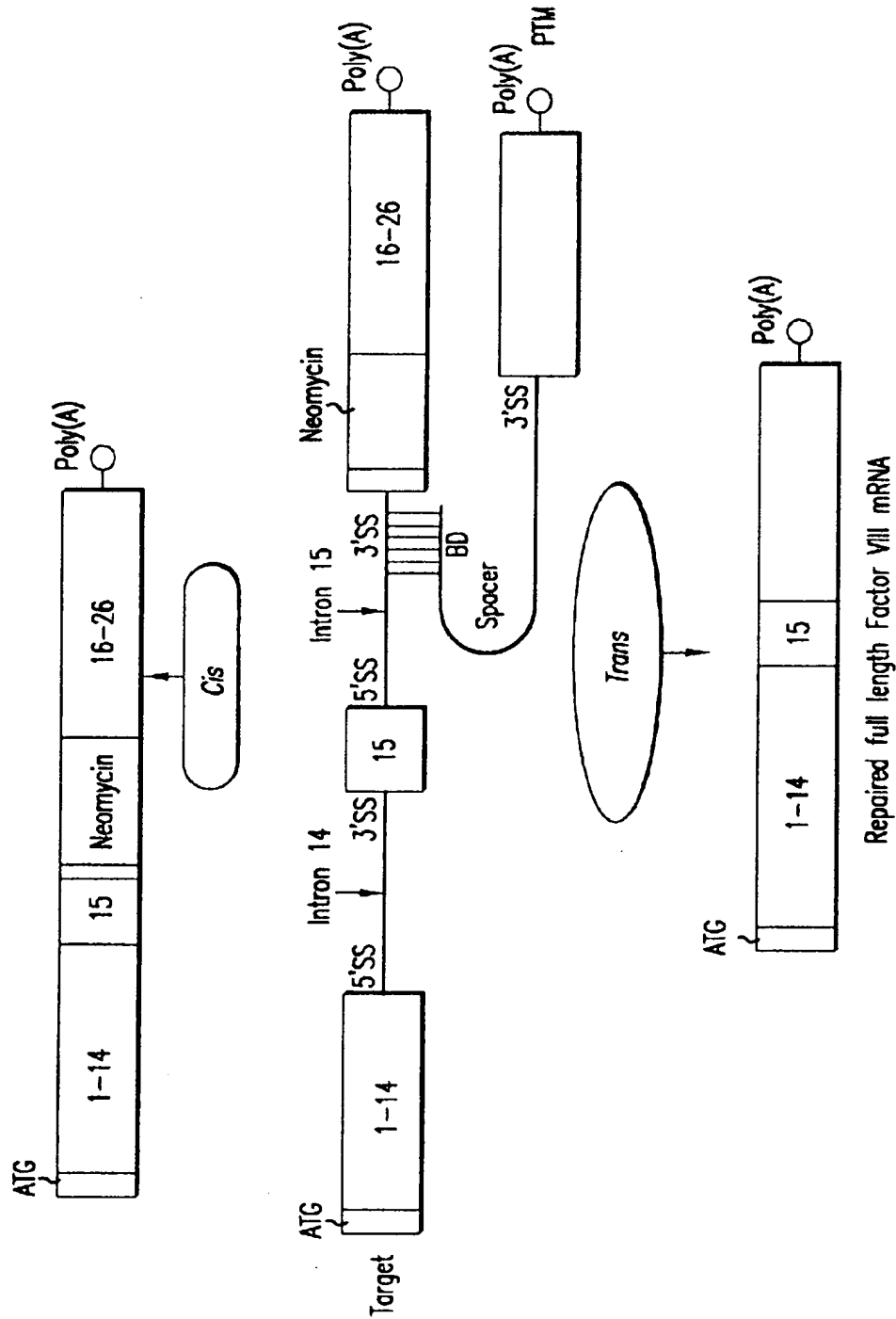
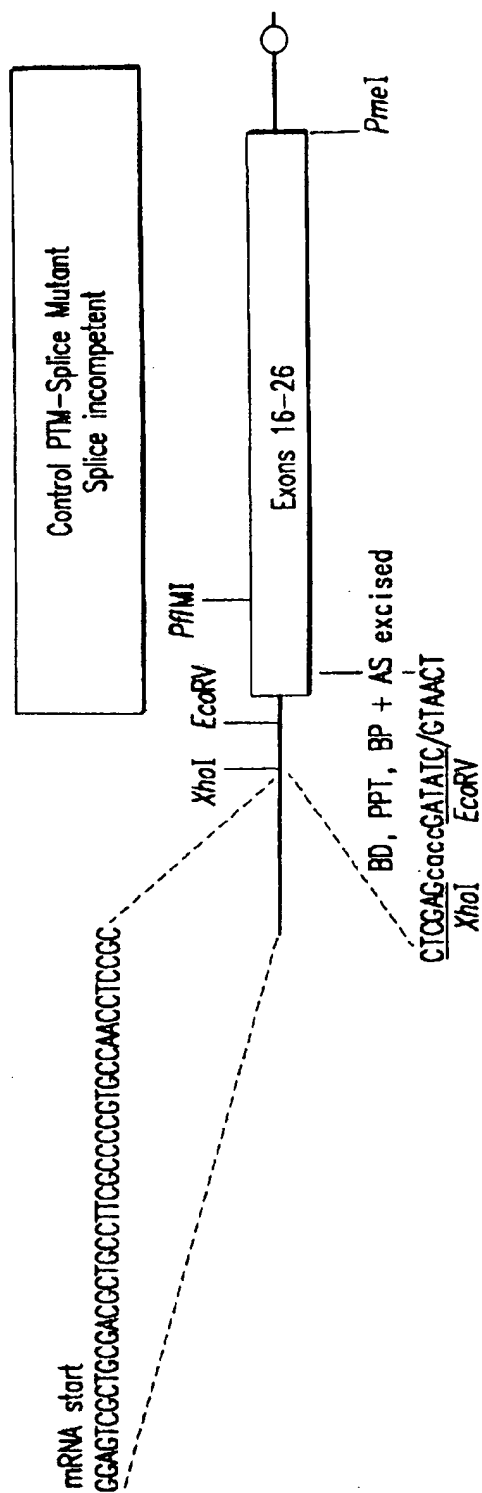


FIG.44D



# Method:

Excise TSD and part of exon 16 with XhoI and PfuMI and ligate in a PCR product that:

- 1) eliminates the TSD and splice acceptor site
- 2) inserts EcoRV adjacent to exon 16
- 3) restores the coding for exon 16

FIG.45

Repair of Factor VIII  
Preliminary results from one experiment

FVIII activity in Exon 16 FVII-KO mice  
after IV PTM-FVII intraportal infusion  
(100 $\mu$ gDNA)(n=3)

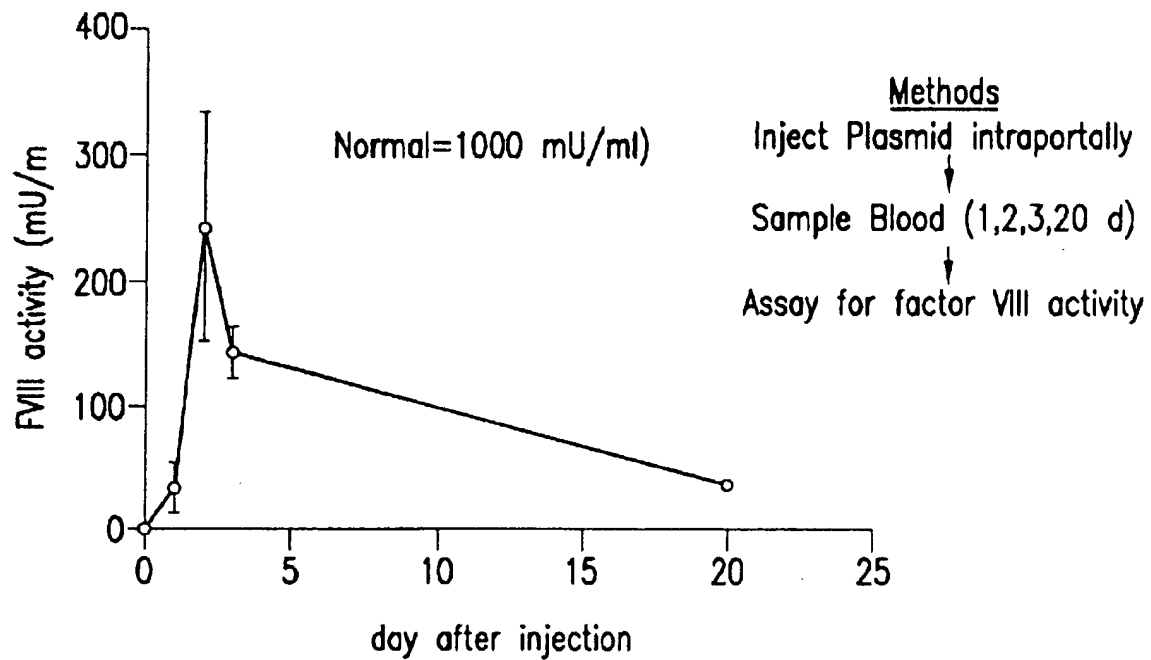


FIG.46



Detailed structure of a mouse factor VIII PTM containing normal sequences for exons 16-26 and a C-terminal FLAG tag. BGH=bovine growth hormone 3' UTR; Binding domain= 125 bp.

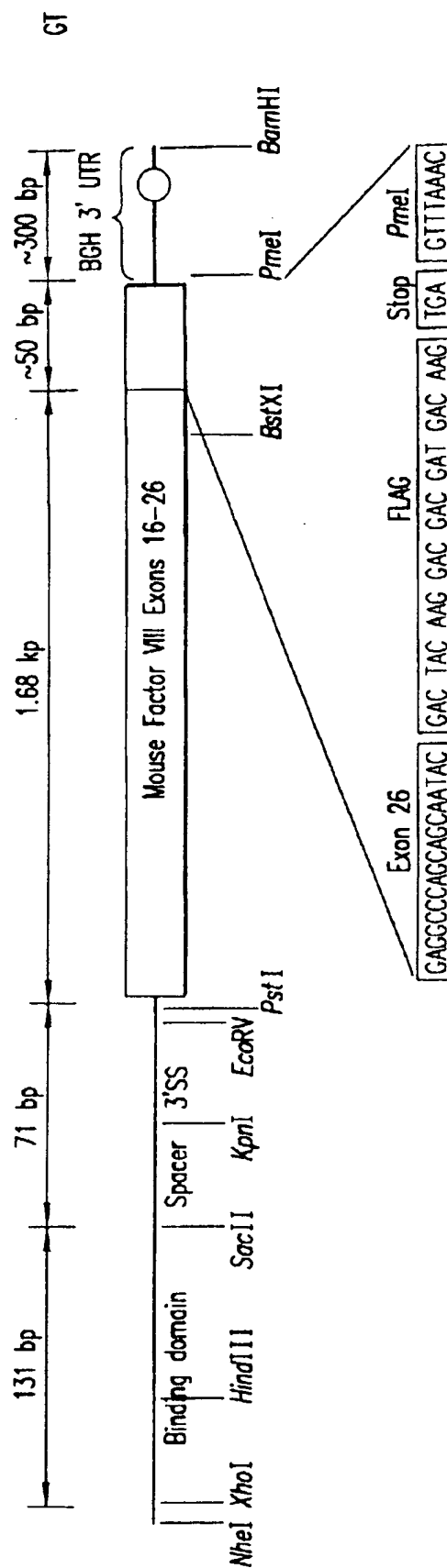
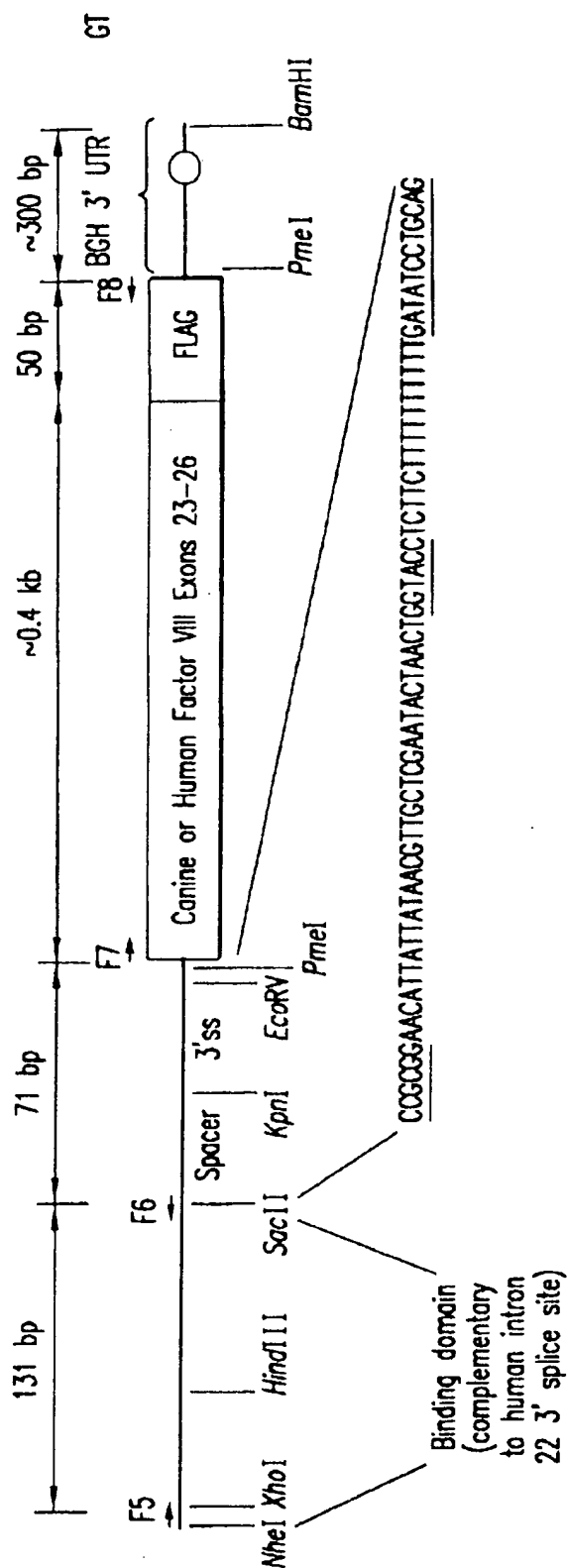


FIG.47A



FLAG=C-terminal tag to be used to detect repaired factor VIII protein.

FIG.47B